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# Comparative anatomy and evolution of the gastrotrich muscular system

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# COMPARATIVE ANATOMY AND EVOLUTION OF THE GASTROTRICH MUSCULAR SYSTEM

BY

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BS, Humboldt State University, 1993

MA, Humboldt State University, 1998

DISSERTATION

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April 8 2002
Date

## DEDICATION

For my parents, Arthur and Sandra Hochberg, whose encouragement and support have been without equal throughout my college career.

I would also like to dedicate this thesis to the memory of the late Dr. Gary J. Brusca. Gary was more than a great Invertebrate Zoologist, he was a great inspiration to thousands of students like myself, and he will be sorely missed but never forgotten.

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ABSTRACT

COMPARATIVE ANATOMY AND EVOLUTION OF THE GASTROTRICH MUSCULAR SYSTEM

By

Richard Hochberg

University of New Hampshire, May, 2002

Gastrotrichs figure prominently in metazoan phylogeny because they share a suite of complex morphological characteristics with several other members of the Bilateria. But their microscopic size, cryptic interstitial habitat, and lack of fossil record have exacerbated the usual barriers to phylogenetic analysis. To arrive at a better understanding of gastrotrich systematics and evolution, cladistic analyses and detailed studies of the muscular system were performed.

A fluorescent F-actin stain was applied to whole mounts of 26 species of Gastrotricha to characterize the musculature. Muscle patterns were mapped, their functions inferred, and the direction of evolution hypothesized for several families. The musculature of all gastrotrichs is arranged as a series of circular, helicoidal, and longitudinal bands around the digestive tract. Circular muscles are generally present in splanchnic and somatic positions. Helicoidal muscles in 50-60° angles are present on the pharynx and intestine of most species. Longitudinal muscles are arranged radially around the digestive tract in dorsal, lateral, ventral and ventrolateral positions. Extraordinary muscle orientations are present in several species.

In macrodasyidan gastrotrichs, the musculature of *Dactylopodola baltica* (Dactylopodolidae) is considered to be closest to the ground pattern of the phylum and consists of the following: splanchnic circular muscles on the pharynx and intestine, longitudinal muscles in dorsal, lateral, ventral and ventrolateral positions, pharyngeal and intestinal helicoidal muscles, and somatic circular muscles. Within the Chaetonotida, species of *Neodasys* and *Xenotrichula* have the most plesiomorphic muscle topologies. Muscle patterns are similar to macrodasyidans though several muscle orientations have become reduced (splanchnic and somatic circular muscles), are the result of evolutionary modification to existing muscles (incomplete splanchnic and somatic circular muscles, dorsoventral muscles) or evolved independently (the branched Rückenhautmuskel).

This study relied on a phylogenetic perspective to delineate the origin of specific muscle patterns in gastrotrichs and allow for the separation of phyletic heritage from adaptation. Several species from both orders possess muscle patterns that can be regarded as apomorphic and may therefore serve as taxonomic characters. Closer scrutiny of these species may reveal the underlying selective processes that led to the origin and maintenance of novel muscle orientations in gastrotrichs.

# INTRODUCTION

The Gastrotricha is a diverse group of microscopic (50 μm – 3500 μm) free-living invertebrates. Marine gastrotrichs inhabit the interstitial mesopsammon and are common in coastal habitats ranging from the upper intertidal to the continental shelf. In coastal regions they may be the numerically dominant interstitial species (Hochberg 1999). Freshwater species are also abundant and may be found in the interstices, epibenthic or on submerged vegetation.

Since their first description by Müller in 1786, gastrotrichs have held an enigmatic status because of their miniscule size and absence of both coelom and segmentation. Zelinka (1889) and Remane (1933, 1936) produced detailed monographs on the phylum, but not until the advent of electron microscopy were peculiar aspects of their morphology made clear and a better understanding of their phylogenetic relationships made evident. The acoelomate status of gastrotrichs was was finally clarified (Teuchert & Lappe 1980; Rieger et al 1974; Ruppert 1991) while various other studies contributed important systematic information and helped to clarify the monophyletic status of the Gastrotricha (Rieger 1976; Rieger & Rieger 1977). Subsequently, superphyletic taxa such as the Aschelminthes (Gröbben 1910) were discarded in favor of other hypotheses on "pseudocoelomate" relationships. Synapomorphies such as the structure of the myoepithelial pharynx (Ruppert 1982) and cerebral ganglion (Neilsen 1995) are currently used to define potential relationships to other taxa. Today, the Gastrotricha recognized as a distinct phylum– few researchers still

regard it as a class closely aligned with other taxa (e.g., Gnathostomulida, Cavalier-Smith 1998).

The Gastrotricha possess several autapomorphies that make it a well-defined taxon: exocuticle of multiple unit-like membranes, cuticularized adhesive tubes, cuticle-covered cilia and egg release by rupture of the body wall (Boaden 1985; Lorenzen 1985). The most recently discovered autapomorphy is found in the ciliary pit of sensory cilia, where ten symmetrically arranged stereocilia are present (Fig.1; Hochberg 2002).

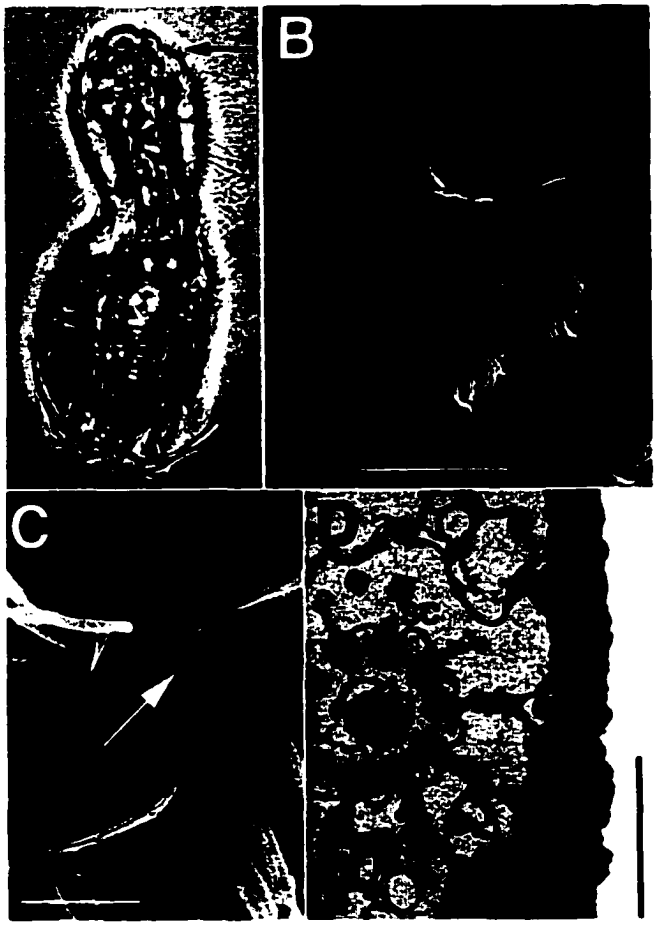


Figure 1. Sensory cilia in *Lepidodermella squamata*. A) Site of cilia. B) SEM of cilia. C) Close-up of cilia base. D) Transverse section through ciliary pit showing cuticle (cu), kinocilium (kc), and stereocilia (sc).

The phylum is traditionally divided into the orders Chaetonotida and Macrodasyida (Fig. 2). Order Chaetonotida is comprised of two suborders, Paucitubulatina and Multitubulatina, both defined by the presence of a pharynx with a Y-shaped lumen. Paucitubulatina contains five families of marine and freshwater gastrotrichs with a ten-pin body shape and a pair of caudal adhesive tubes (Fig. 3). Members of the order are often extremely small (80 μm – 500 μm) with a highly ornamented cuticle and a life-cycle with a parthenogenetic phase. The Multitubulatina is considered an enigmatic taxon with characteristics of both Paucitubulatina and Macrodasyida. The suborder contains only a single monogeneric family, Neodasyidae, consisting of three marine species that possess multiple adhesive tubes like macrodasyidan gastrotrichs, but with a pharynx lumen and innervation similar to the chaetonotidans (Ruppert 1991). Macrodasyidan gastrotrichs are often more vermiform (150 μm to 3500 μm) than chaetonotidans, and have a pharynx with an inverted Y-shaped lumen and pharyngeal pores. Macrodasyidans also possess complex reproductive organs and adhesive tubes distributed in anterior, lateral, and posterior series. Most macrodasyidans are marine interstitial forms with only a single genus known from freshwater (Kiselewski 1987).

The morphology of gastrotrichs at the level of light microscopy has provided important clues on intraphyletic relationships (Travis 1983), but morphology is often highly variable, even within species, possibly due to ecological factors (Hummon 1969). Comparative ultrastructural analyses have thus been performed on various organ systems such as the cuticle (Rieger & Rieger 1977), body-wall (Rieger 1976; Travis 1983), pharynx (Ruppert 1991), and reproductive organs (Ruppert 1978a,b) to elucidate

inconsistencies in taxonomic classification (e.g., taxonomic status of *Chordodasys*, Hummon 1974) and evolutionary relationships (e.g., position of *Lepidodasys* relative to Thaumastodermatidae, Ruppert 1978b). While ultrastructural studies provide useful functional and phylogenetic information, they often bypass larger structural features not evident in sectional view. Therefore, an ultrastructural analysis represents only one level of study within a structural hierarchy of an organism that can be used to understand phylogenetic patterns. Another line of evidence, and one often neglected, is the topology of various organ systems. Growing evidence exists to support the view that the topology of the muscular system can provide useful characters for taxonomy (Tyler & Hyra 1998; Hooge & Tyler 1999a) and phylogeny (Hooge & Tyler 1999b; Hooge 2001).

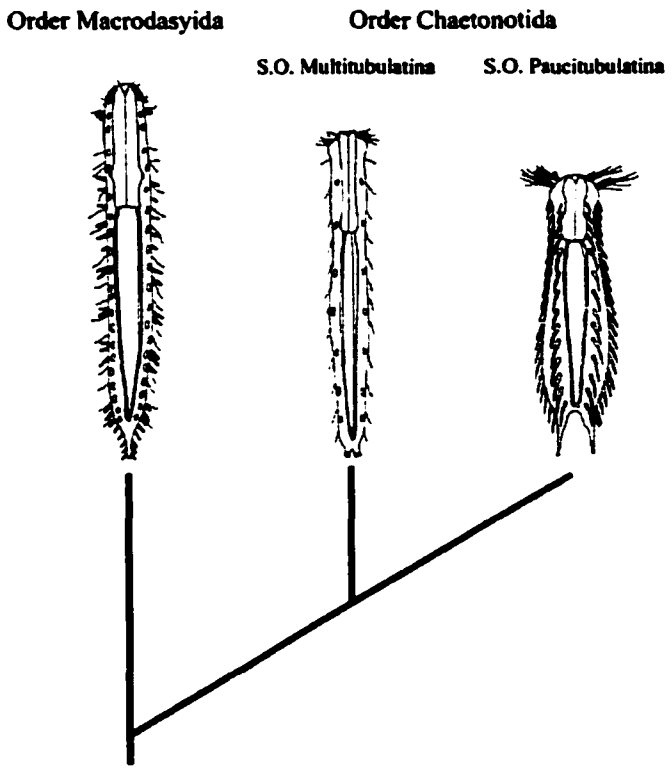


Figure 2. Traditional phylogenetic classification of the Gastrotricha.

The gastrotrich muscular system is generally defined as a coaxial system of individual muscle bands, present in circular and longitudinal orientations (Ruppert 1991). Circular muscles are organized as hoop-like rings around the gut tube, occasionally sending out lateral branches to encompass other muscle groups or reproductive organs. Longitudinal muscles are present as individual bands, sometimes grouped closely in parallel as a single functional unit, and generally extending from the pharynx to the caudal end. Aside from the presence of dorso-ventral muscles in some species, and the tendency to reduce circular muscles in various clades, the gastrotrich muscular system is assumed uniform at the gross anatomical level (Ruppert 1991). Variation is presumably more prevalent in muscle ultrastructure, seen best in myocyte shape, sarcomere organization, and the structure of the excitation-contraction coupling system (Ruppert 1991).

Morphological assessments of intraphyletic relationships have made use of muscle characteristics in gastrotrichs (Ruppert 1982; Travis 1983), showing that the muscular system has phylogenetic utility. The goal of this study is to address this issue of phylogenetic utility using the gross anatomy of the muscular system as a source of phylogenetic characters. While an exhaustive systematic survey of the Gastrotricha is beyond the scope of this study, numerous species from each order, representing five families of Macrodasyida and five families of Chaetonotida, were examined in detail. This study also addresses functional morphology of the gastrotrich muscular system, since any evolutionary change in muscle orientation is likely to create corresponding shifts in functional relationships.

The detailed and comparative nature of this study necessitates its division into nine chapters, beginning with the current assessment of gastrotrich phylogeny based on morphology (Hochberg & Litvaitis 2000c). Chapter 2 presents a more detailed cladistic analysis of the Macrodasyida with emphasis on defining higher-level taxa (families) and character transformations within the order (Hochberg & Litvaitis 2001c). Chapters 3 is a comparative analysis of the macrodasyidan muscular system, synthesizing information derived from muscle topology into character descriptions and presenting it in a phylogenetic perspective (Hochberg & Litvaitis 2001d). Chapter 4 presents a descriptive analysis of the gastrotrich, *Tetranchyroderma papii*, to highlight functional aspects of specific muscle patterns in macrodasyidans (Hochberg & Litvaitis 2001b). Chapter 5 describes a new muscle orientation in gastrotrichs, helicoidal muscles, focusing on both functional and phylogenetic aspects (Hochberg & Litvaitis 2001a). Chapter 6 presents a detailed analysis of the chaetonotidan muscular system (Hochberg & Litvaitis 2002), followed by a description of the muscles of *Draculicteria tessalata* (Hochberg & Litvaitis 2001e) in Chapter 7 and *Neodasys australiensis* in Chapter 8, as examples of phylogenetically distant taxa within the order. The last chapter assesses the evolution of the muscular system within the phylum, and attempts to estimate the ground pattern of the Gastrotricha to better evaluate its origin and potential sister group relations to other phyla.

# CHAPTER 1

## PHYLOGENY OF GASTROTRICHA:

## A MORPHOLOGY-BASED FRAMEWORK OF GASTROTRICH RELATIONSHIPS[1]

### Introduction

The currently accepted classification of the phylum Gastrotricha is based on morphological features (Hummon 1982; Ruppert 1988) and has two monophyletic orders, the Macrodasyida and Chaetonotida. Both orders are defined primarily by the structure of the myoepithelial pharynx (Ruppert 1991), yet several other characteristics are important in the systematic classification of genera (Hummon 1982; Ruppert 1988; Ruppert 1991) (Fig. 1.1).

Accepted morphological homologies supporting gastrotrich monophyly include the presence of unique, cuticle-covered duo-gland adhesive organs (Tyler and Rieger 1980), a multilayered epicuticle (Rieger and Rieger 1977) and cuticle-covered locomotory and sensory cilia (Rieger and Rieger 1977). Other features often used to categorize gastrotrichs (cross-striated muscles (Travis 1983), monociliated epidermis (Rieger 1976), triradiate myoepithelial pharynx (Schmidt-Rhaesa et al. 1998) and bilayered nature of the cuticle (Schmidt-Rhaesa et al. 1998) are plesiomorphies and

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1. Hochberg R. & Litvaitis, M.K. 2000c. Phylogeny of Gastrotricha: a morphology-based framework of gastrotrich relationships. Biological Bulletin 198: 299-305.

therefore, of limited systematic value. Of particular significance is the presence of a monociliated epidermis; this condition is considered a primitive trait among Metazoa (Rieger 1976). A monociliated epidermis is found in several gastrotrich taxa (Rieger 1976), including the Dactylopodolidae and Neodasyidae, arguing further for their basal positions within their respective orders. That the order Chaetonotida may be paraphyletic with respect to the phylum Nematoda, as suggested previously (Ruppert 1982) is indicated by similarities in cuticular and pharyngeal ultrastructure (upright Y-shaped lumen, pharyngeal intestinal valve), and pharyngeal innervation. Because of the complete absence of locomotory cilia in Nematoda, in conjunction with several other morphological characters (Lorenzen 1985), we agree with Lorenzen (1985) that the Nematoda is at best, a sister taxon to the Gastrotricha.

## Material and Methods

We have analyzed relationships of nearly all known gastrotrich genera and evaluated them using parsimony analysis (PAUP 4.0*; Swofford 1999). Eighty one characters were used in the analysis, all unordered and unweighted (Table 1). For maximum parsimony, default settings included ACCTRAN and multistate taxa were treated as uncertainties. Bootstrap options included 1000 replicates of 81 characters. Full heuristic searches were performed with starting trees obtained by stepwise random addition (10 replicates with 2 trees held at each step). Tree bisection-reconnection (TBR) branch swapping was performed with the MULTREES option to save all minimum-length trees. Two genera, *Marinellina* and *Undula,* were excluded from the analysis due to a lack of information. The Gnathostomulida and Nematoda were used as outgroups.

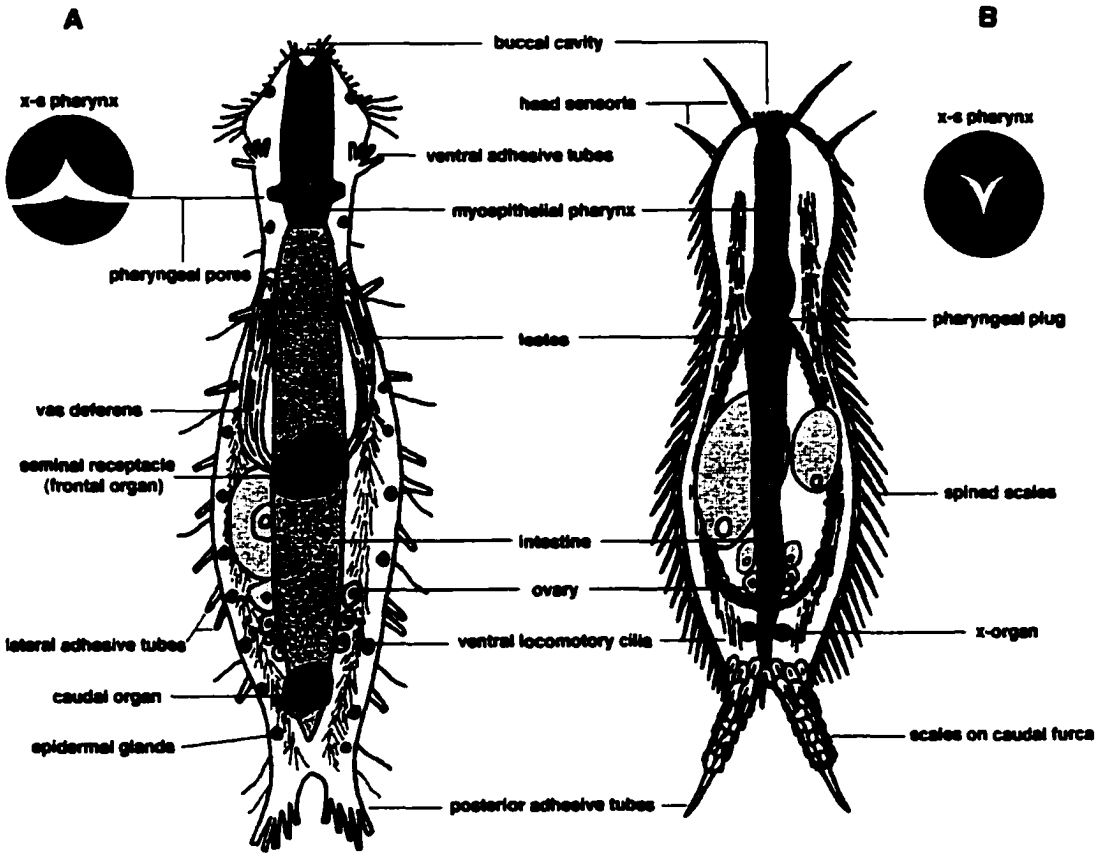


Figure 1.1. Schematic representation of (A) a macrodasyidan and (B) a chaetonotidan gastrotrich, showing characters separating the two orders. Cross-sections through pharynges show orientation of lumen and in the macrodasyidan, the pharyngeal pores.

Table 1.1 Characters used in the phylogenetic analysis. A= absent, p = present.

---

1. Shape of myoepithelial pharyngeal lumen (0 = a, 1 = Y-shape, 2 = upside-down Y-shape)
2. Pharyngeal clefts (0 = a, 1 = p)
3. Epidermal glands (0 = a, 1 = p)
4. Cuticularized adhesive organs (tubes) (0 = a, 1 = p)
5. Paired ova mature in anterior to posterior direction (0 = a, 1 = p)
6. Posterior nerve cords leaving circumpharyngeal ganglion (0 = multiple, 1 = 2 total)
7. Epidermal ciliation (0 = monociliation, 1 = multiciliation, 2 = no ciliation)
8. Y-cells derived from longitudinal muscle block (0 = a, 1 = p)
9. Lateral adhesive tubes - duogland type (0 = a, 1 = p)
10. Ventrally placed anterior adhesive tubes (0 = a, 1 = p)
11. Scales constructed from basal cuticular layer - form solid endocuticular thickenings (0 = a, 1 = p)
12. Posterior adhesive tubes (0 = a, 1 = p)
13. Muscle striation pattern (0 = X-Striated, 1 = Oblique)
14. Sperm ducts join caudal organ (0 = a, 1 = p)
15. Seminal receptacle (frontal organ) (0 = a, 1 = p)
16. Caudal organ (penis) (0 = a, 1 = p)
17. Cephalic chemoreceptors present as piston pits, pestles, cones or palps (0 = a, 1 = p)
18. Cuticular hooks/ancres (triancres, tetrancres, pentacres) (0 = a, 1 = p)
19. Myoepithelial pharynx with cilia (0 = a, 1 = p)
20. Ventrally placed extraordinary adhesive tubes as Seitenfüsschen (0 = a, 1 = p)
21. Complex frontal and caudal organs separate & without tissue connection (0 = connection present, 1 = connection absent, 2=inapplicable)
22. Vas deferens (0 = absent, 1 = separate vas deferens - paired or unpaired, 2 = always paired and often fused)
23. Microvilli penetrate pharynx exocuticle (0 = a, 1 = p)
24. Ciliated gut epithelium (0 = a, 1 = p)
25. Y-cell with myofilaments (0 = a, 1 = p)
26. Rosette organ (0= a, 1 = p)
27. Rounded caudal end bearing numerous adhesive tubes (0= a, 1 = p)
28. Caudal end with tail-like extension bearing adhesive tubes (0 = a, 1 = p)
29. Caudal end with elongate peduncle bearing adhesive tubes (0 = a, 1 = p)
30. Caudal end is biramous/forked (0 = a, 1 = p)
31. Anterior adhesive tubes in arc-like orientation, never as distinct clumps in common base (0 = a, 1 = p)
32. Anterior adhesive tubes on a mobile elevated base (0 = a, 1 = p)

Table 1.1 continued.

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33. Anterior adhesive tubes arranged together - as hands or clumps (0 = a, 1 = p)
34. Lumen of caudal and frontal organs continuous (0 = a, 1 = p)
35. Wide flaring buccal region (0 = a, 1 = p)
36. Small pharyngeal pores (0 = a, 1 = p)
37. Paired testis (0 = otherwise, 1 = p)]
38. Reduced left testis - single right testis only (0 = otherwise, 1 = p)
39. Reflexed vas deferens ( 0 = a, 1 = p)
40. Epidermal glands with band-like contents (0 = a, 1 = p)
41. Distinct head enclosing most of the pharynx (0 = a, 1 = p)
42. Proximal/distal separation of caudal adhesive tubules on elongate peduncle (0 = a, 1 = p)
43. Seitenfüsschen as paired "brocha" tubules (0 = a, 1 = p)
44. Seitenfüsschen as single "cirrata" tubules (0 = a, 1 = p)
45. Median caudal cone (0 = a, 1 = p)
46. Single pair of posterior head cones (0 = a, 1 = p)
47. Circular muscles absent from lateral body regions (0=p, 1 = a)
48. At least one pair of "soft, palp-like organs" on head (0 = a. 1 = p)
49. One pair of short "cirri" (0 = a, 1 = p)
50. Lateral adhesive organs indistinguishable, present as minute papillae (0 = absent, 1 = present)
51. Parthenogenesis (0 = a, 1 = p)
52. Ventral cilia and head sensoria modified as cirri (0 = a, 1 = p)
53. Pharyngeal intrusions "teeth" ( 0 = a, 1 = p)
54. Cirri construction (0 = a, 1 = constructed as a singular unit, 2 = each cilium with own cuticle)
55. Circular muscles (0 = p, 1 = a)
56. Spines sculpted from surface zone of basal cuticle layer (not originating from flat scales) (0 = a, 1 = p)
57. Club-shaped tentacles on head (0 = a, 1 = p)
58. Bifid caudal end with 2 posterior adhesive tubules (0 = absent, 1 = present)
59. U-shaped gonad with caudal anastomosis (0 = a, 1 = p)
60. Keeled scales derived from surface-zone of basal cuticular layer (0 = a, 1 = p)
61. Ventrolateral hydrofoil scales derived from surface-zone of basal cuticular layer (0 = a, 1 = p)
62. Surface zone (of basal layer) spines present in groups (0 = a, 1 = p)
63. Stylochaeta-type ciliation ( 0 = a, 1 = p)
64. Ten-pin body shape (0 = elongate, 1 = ten-pin)

Table 1.1 continued.

65. Pharyngeal foldings/plug (0 = a, 1 = p)
66. Surface-zone (of basal layer) scales (0 = a, 1 = p)
67. X-organ (0 = a, 1 = p)
68. Head plates (0 = a, 1 = p)
69. Modified buccal capsule (0 = simple opening, 1 = extended/folded/etc)
70. Surface-zone (of basal layer) scales lacking stalk and endplate (0 = a, 1 = p)
71. Elongate scale-covered furca (0 = a, 1 = p)
72. Number of longitudinal tracts of pharyngeal nerves (0 = >3 nerves, 1 = 3 nerves)
73. Ventral locomotory cilia restricted to head region as a distinct patch (0 = a, 1 = p)
74. Caudal furca reduced to bilateral protuberances without adhesive tubes (0 = otherwise, 1 = p)
75. Ventral locomotory cilia present in 2 longitudinal rows (0 = a, 1 = p)
76. Longitudinal rows of locomotory cilia abbreviated at mid-body region (0 = otherwise, 1 = p)
77. Vas deferentia (0 = elongate, 1 = reduced/absent)
78. Cilia covered with cuticle (0 = a, 1 = p)
79. Testes present as bilateral packets in post-parthenogenetic phase (0 = otherwise, 1 = p, 2 = no testes)
80. Surface-zone (of basal layer) scales in ventral interciliary field (0 = a, 1 = p)
81. Surface-zone (of basal layer) scales in ventral interciliary field reduced to terminal field (0 = a, 1 = p)

## Results

The analysis resulted in a monophyletic Gastrotricha within which each order formed a monophyletic clade (Fig. 1.2). Although the Macrodasyida is a highly heterogeneous group defined primarily by pharyngeal structure (Ruppert 1991), the presence of groups of adhesive tubules (Hummon 1982; Ruppert 1988), and curious reproductive organs (Ruppert and Shaw 1977; Ruppert 1978), the analysis confirmed several monophyletic taxa within the order (Fig. 1.2). In fact, characters of the latter two organ systems substantiate monophyly of the two ecologically most diverse families, the Turbanellidae and Thaumastodermatidae (Fig. 1.2). These two families occur in nearly all marine environments at all depths, inhabit a wide array of sand types, and are probably among the most ubiquitous and successful groups of interstitial animals. As noted previously (Ruppert 1988), the Lepidodasyidae is an unnatural taxon difficult to define on current morphological criteria. The analysis found a paraphyletic Lepidodasyidae with some genera clustering with other families, although with low bootstrap values (Fig. 1.2). The Dactylopodolidae is affirmed as the most primitive macrodasyidan family, with retention of several plesiomorphic traits including epidermal monociliation, alimentary ciliation, cross striated muscles and separate multiple nerve cords in some genera (Fig. 1.2). Additional research on this family, in particular the relatively unknown *Dendrodasys* and *Dendropodola*, should further elucidate the ground pattern for the Gastrotricha.

Bootstrap values advocating a monophyletic Chaetonotida are relatively weak (<50 %), but values for the monophyly of the suborder Paucitubulatina are more robust (94%) (Fig. 1.2). All chaetonotidans are largely defined by the configuration of the

pharyngeal lumen (Y-shaped) and the absence of pharyngeal pores, but often body-shape ("ten-pin") combined with the absence of anterior or lateral adhesive tubules are the most easily seen diagnostic characters. The monogeneric Multitubulatina, however, contains an anomalous vermiform *Neodasys* that shares traits with both Macrodasyida and Paucitubulatina. However, unlike all other members of the Chaetonotida, *Neodasys* is highly elongate and in possession of some potentially plesiomorphic characteristics: hermaphroditic gonads, complex reproductive organs, monociliated epidermis, smooth cuticle, and numerous adhesive tubules (Ruppert 1991). Furthermore, the adhesive organ of *Neodasys* lacks a releaser gland (Tyler et al. 1980). The presence of only one gland type in the adhesive organ is interpreted as a plesiomorphy when viewed in combination with the retention of other primitive traits (Tyler et al. 1980). Still, the presence of dual-gland adhesive organs in all Gastrotricha has not been confirmed, and it remains to be seen whether the presence of only a single gland is actually the 'primitive' condition or secondarily derived.

Within the Paucitubulatina, the largest and structurally most diverse family, the Chaetonotidae, appears to be polyphyletic (Fig. 1.2). This highly speciose taxon (> 250 spp.) is extremely heterogeneous with respect to cuticular armature, habitat type, and reproductive anatomy. The family is presumed to have evolved in the freshwater benthos, radiating into the marine environment secondarily (Kisielewski 1990). Yet, the existence of a very basal marine genus, *Musellifer*, might also argue for a marine origin. Resolution of this vastly diverse taxon will undoubtedly improve with greater attention to their reproductive anatomy (presence of hermaphroditic organs is unknown for many genera; the homology of the X-organ is in question) and the microstructure of scales and spines.

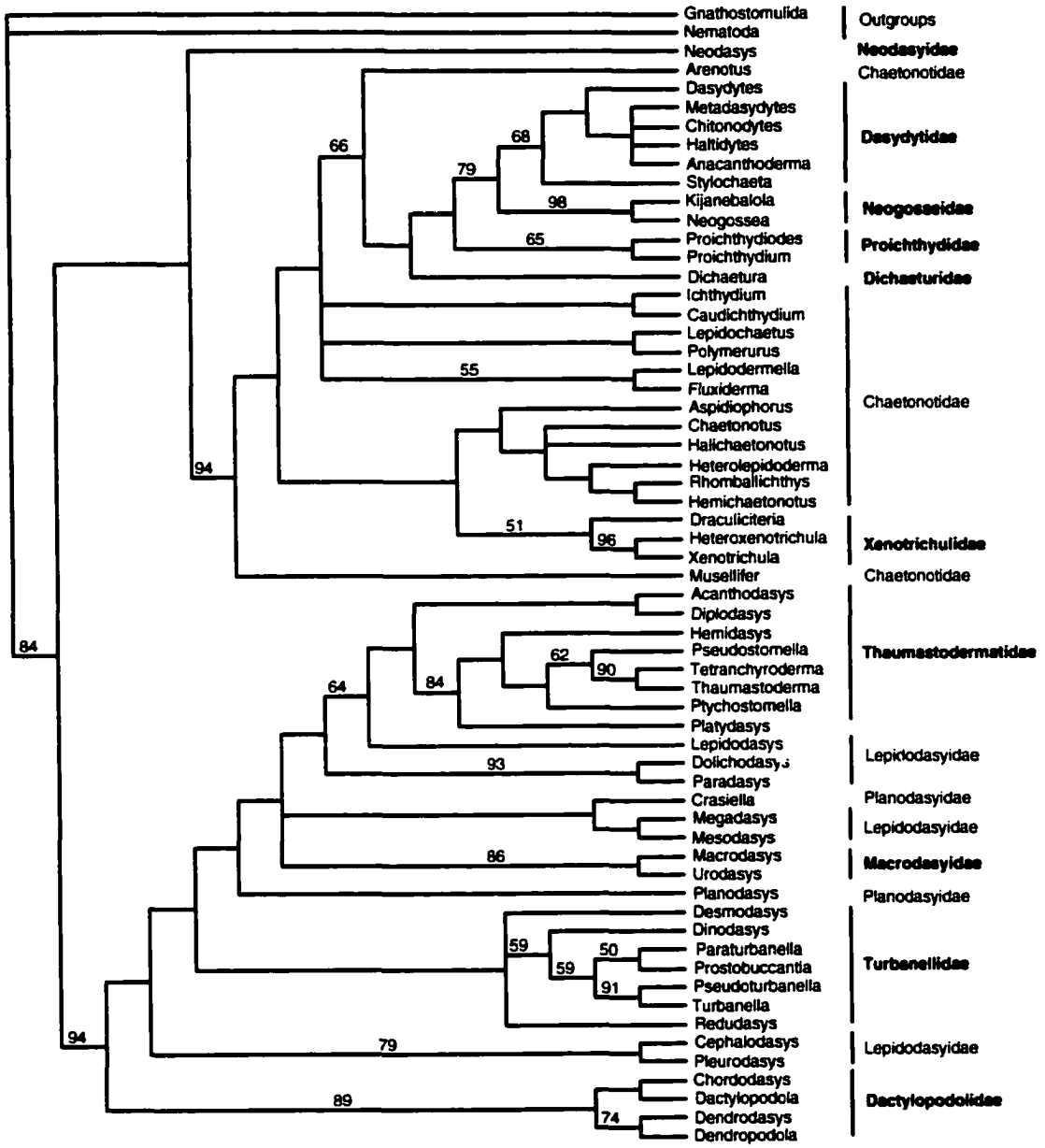


Figure 1.2. Consensus tree (50% Majority-Rule ) of 59 gastrotrich genera obtained using PAUP version 4.0b1 for Macintosh. Tree length = 175 steps; CI = 0.503, RC = 0.450. Numbers at nodes represent percentages of 1000 bootstrap replications. Family names in bold are monophyletic according to this analysis.

Still, several important monophyletic groupings were confirmed in this analysis and included the entirely freshwater Dasydytidae, Neogosseidae and Proichthydidae (Fig. 1.2). The analysis thus confirmed a clade of freshwater families with 79% of bootstraps. In addition, the marine family Xenotrichulidae and the subfamily Xenotrichulinae were also monophyletic (Fig. 1.2).

## Discussion

The current analysis goes beyond classification; it is aimed at an elucidation of phylogenetic trends. In particular, trends in nervous-system structure in the Gastrotricha are revealed within the tree topology of the Macrodasyida. Basal genera often have multiple, separate nerve cords (4 in *Dactylopodola*), while more derived genera show partial fusion of nerve cords (4 > 2 in *Turbanella*) or presumably complete fusion (2 in Thaumastodermatidae). The functional significance of this transition series is unknown; moreover, the trend is more ambiguous than supposed since other 'intermediate' forms like *Cephalodasys maximus* show fusion ("schmelzen") of multiple (12) nerve cords, followed by subsequent bifurcation (Wiedermann 1995). Other trends are also evident. For example, changes in reproductive biology (from hermaphroditic to parthenogenetic) seem to have occurred in chaetonotidans transitions from marine to freshwater biotopes.

This cladistic analysis also provides evidence that many characters used to define gastrotrichs in morphology-based phylogenies of extant Metazoa often are apomorphic for the phylum and therefore, inappropriate. For example, Wallace et al. (1996) use the characters 'parthenogenesis and syncytial epidermis' in their analysis. These chracters though are known only in some chaetonotidans. Additionally, these authors use the

character of hypodermic impregnation (Wallace et al. 1996). However, it is unconfirmed except for the family Macrodasyidae and it is unknown in basal taxa such as Dactylopodolidae. Backeljiau et al. (1993) incorrectly have used eutely (found only in some chaetonotidans), and radial cleavage (gastrotrichs have aberrant bilateral cleavage) as characters defining gastrotrichs as a whole in their analysis. Finally, Zrzay et al. (1998) used the character 'pseudocoelomate' in their analysis, although all gastrotrichs are acoelomate.

The cladistic analysis also provides a good test for molecular phylogenetic studies. Several earlier molecular studies have focused on derived taxa (*Chaetonotus* sp., *Lepidodermella squamata*) to resolve phylum-level relationships (Winnepenninckx et al. 1995; Littlewood et al. 1998; Zrzavy et al. 1998; Ruiz-Trillo et al. 1999). Such taxa are particularly easy to culture, but the utility of these taxa as representative models is questionable. *Lepidodermella squamata* is especially controversial because it inhabits freshwater and is parthenogenic. Characteristics that are uncommon among chaetonotidans and virtually absent in macrodasyidans. The use of such derived forms should be avoided until the systematics of the Chaetonotida is better resolved.

Additionally, molecular systematics place the Gastrotricha in a variety of positions within the metazoan tree: as a sister group to either the Acanthocephala (Carranza et al., 1997), to the Gnathostomulida (Littlewood et al., 1998), to the Nematomorpha (Carranza et al. 1997), to the Platyhelminthes (Winnepenninckx et al. 1995). Other studies place the Gastrotricha basal to most of the Bilateria (Carranza et al. 1997), or the Lophotrochozoa (Ruiz-Trillo et al. 1999), or the Spiralia (Littlewood et al. 1998).

We contend though, that the position of the Gastrotricha remains questionable until such time as more primitive gastrotrich species are utilized, namely *Neodasys* or species of the Dactylopodolidae. These additions to the molecular data set would be a good test of tree strength and confirmation their branching point from the remaining phyla.

A comprehensive and congruent classification of the phylum Gastrotricha is essential if its origin and phylogenetic significance is to be fully appreciated. The currently accepted classification is in dire need of revision, especially concerning the order Chaetonotida and the family Lepidodasyidae. At the same time though, it is important to note that previous work on the systematics of the phylum has successfully navigated the treacheries of convergence and parallelism and displayed good phylogenetic congruence (Remane 1933; Hummon 1982; Ruppert 1988). Yet, despite the rather small size of the phylum, the species are highly variable in particular characters (e.g., adhesive tubes, ciliary patterns, cuticular sculpture, reproductive anatomy, etc.) and recognition of plesiomorphy is often difficult. Morphology will continue to play a key role in the greater understanding of this enigmatic phylum, and future work on gastrotrich systematics should clarify many of the more problematic issues (homology of reproductive organs, number of nerve cords and extent of fusion) through increased use of electron microscopy and immunocytochemistry. Still, there is a great need to accumulate additional molecular data about the more primitive genera, and only then will we achieve a more coherent understanding of relationships within the phylum and its relationships to other animals.

Table 1.2. Data matrix used in phylogenetic analysis.

**Outgroups**

Gnathostomulida 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 2 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Nematoda 1 0 0 0 0 0 2 0 0 0 0 0 1 0 0 0 0 0 1 0 2 2 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0
0 0 1 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0

**Order Macrodasyida**

Acanthodasys 2 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 0 0 1 0 0 1 1 1 0 0 0 1 0 0 0 1 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Cephalodasys 2 1 1 1 0 0 0 1 1 1 0 1 1 0 1 1 ? 0 0 0 0 1 0 0 0 0 1 0 0 0 0 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Chordodasys 2 1 1 1 0 1 0 0 1 1 0 1 0 0 1 1 0 0 1 0 0 1 1 1 0 0 0 0 1 1 0 1 1 0 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Crasiella 2 1 1 1 0 1 0 0 1 1 0 1 1 0 1 1 1 0 0 0 0 2 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Dactylopodola 2 1 1 1 0 0 0 0 1 1 0 1 0 0 1 1 0 0 1 0 0 1 1 1 0 0 0 0 1 1 0 1 1 0 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Dendrodasys 2 1 1 1 0 ? 0 0 0 1 0 1 0 0 1 1 0 0 1 0 0 1 0 1 0 0 0 0 1 1 0 1 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Dendropodola 2 1 1 1 0 ? 0 0 1 1 0 1 0 0 ? ? 0 0 1 0 0 1 0 ? 0 0 0 0 1 1 0 1 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Desmodasys 2 1 1 1 0 ? 1 ? 0 1 0 1 ? 0 ? 1 1 0 0 0 0 2 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Dinodasys 2 1 1 1 0 ? 1 ? 1 1 0 1 ? 0 ? 1 1 0 0 0 ? 2 0 0 0 0 0 0 0 1 0 1 1 0 0 0 1 0 ? 1 0 0 0 0 1 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Diplodasys 2 1 1 1 0 1 1 ? 1 1 1 1 ? 1 0 1 ? 0 0 0 0 1 0 0 0 1 0 0 0 1 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Dolichodasys 2 1 1 1 1 1 1 0 0 1 0 1 1 0 1 1 0 0 0 0 0 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Hemidasys 2 1 1 1 0 1 1 ? 1 1 1 1 ? 1 1 1 ? 0 0 0 0 1 0 0 0 0 0 0 0 1 1 0 0 1 0 1 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 1 0 0 0 1 0 0 0

Lepidodasys 2 0 1 1 0 1 1 1 1 1 1 1 1 1 0 1 ? 0 0 0 0 0 1 0 0 1 0 1 0 0 0 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0

Macrodasys 2 1 1 1 0 1 0 1 1 1 0 1 1 0 1 1 1 1 0 0 0 1 1 0 0 0 0 0 1 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0

Table 1.2. Continued.

Megadasys 2 1 1 1 0 ? 0 ? 1 1 0 1 ? 0 1 1 1 0 0 0 ? 2 0 0 0 0 1 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 ? 0 0 1 0 0 1 0 0 0

Mesodasys 2 1 1 1 0 1 0 0 1 1 0 1 1 1 0 1 1 0 0 0 ? 2 0 0 0 0 1 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0

Table 1.2. Continued.

**Order Chaetonotida**

Neodasys 1 0 1 1 0 0 0 0 0 0 0 1 0 0 1 1 0 0 0 0 ? 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0 0 1 0 1 1 0 0 0

Anacanthoderma 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 1 1 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Arenotus 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0 1 0 1 0 0 1 0 0 0 0 0 1 1 2 1 1 1 0 0 ? 0 0 1 0 1 1 1 0 0

Aspidiophorus 1 0 0 1 0 ? 1 0 0 0 0 1 1 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0 1 1 0 0 1 1 1 1 1 1 0 0 ? 0 0 1 0 1 1 1 1 0

Caudichthydium 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 ? 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 1 0 0 0 0 0 1 1 1 ? 1 ? 0 0 ? 0 0 1 0 1 1 1 1 1

Chaetonotus 1 0 0 1 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 1 0 1 0 0 0 0 1 0 1 1 0 0 1 1 1 1 1 1 0 0 1 0 0 1 0 1 1 1 1 0

Chitonodytes 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0 1 1 0 0 0 0 0 1 0 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Dasydytes 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0 1 1 0 1 0 0 0 1 1 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Dichaetura 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0 1 0 0 1 0 0 0 0 0 0 1 1 0 ? 1 1 0 0 ? 0 0 1 0 1 1 2 0 0

Draculiciteria 1 0 0 1 0 1 1 0 0 0 0 1 0 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 1 0 2 1 0 0 1 0 0 1 0 0 1 1 1 1 1 1 0 0 ? 0 0 1 1 1 1 1 1 0

Fluxiderma 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0 0 0 0 0 1 1 1 ? 1 1 1 0 ? 0 0 1 0 1 1 1 1 0

Halichaetonotus 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 1 0 1 0 0 1 0 1 1 0 0 1 1 1 1 1 1 0 0 1 0 0 1 0 1 1 1 1 0

Haltidytes 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 1 1 0 0 0 0 0 1 1 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Hemichaetonotus 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 1 0 1 0 0 1 0 1 1 0 0 1 1 1 1 1 1 0 0 ? 0 0 1 0 1 1 1 1 0

Heterolepidoderma 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 1 0 1 0 0 1 0 1 1 0 0 1 1 1 1 1 1 1 0 ? 0 0 1 0 1 1 1 1 0

Heteroxenotrichula 1 0 0 1 0 1 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 ? 1 0 1 ? 0 0 1 1 0 1 0 0 1 1 1 ? 1 1 0 0 ? 0 0 1 1 1 1 0 1 0

Ichthydium 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1 0 0 0 ? 0 0 1 0 0 0 0 0 1 1 1 1 1 1 0 0 ? 0 0 1 0 1 1 1 1 1

Table 1.2. Continued.

Kijanebalola 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0
1 0 0 0 1 0 1 0 0 0 0 1 0 1 1 1 ? 0 1 0 0 ? 0 1 0 0 1 1 2 0 0

Lepidochaetus 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 ? 0 0 0 0 0 0 0 0 0 1 0 0 0
1 0 0 0 1 0 0 1 0 1 0 0 0 1 1 1 1 1 1 0 0 ? 0 0 1 0 1 1 1 1 0

Lepidodermella 1 0 0 1 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0
0 1 0 0 0 1 0 0 1 0 0 0 0 0 1 1 1 1 1 1 1 0 1 0 0 1 0 1 1 1 1 0

Metadasydytes 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 1 0 0 0 1 1 0 0 0 0 0 1 1 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Musellifer 1 0 0 1 0 ? 1 0 0 0 0 1 0 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0
1 0 ? 0 0 1 0 1 0 0 0 1 1 1 1 0 1 0 1 1 0 0 1 0 1 1 1 1 0

Neogossea 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1
0 0 0 1 0 1 0 0 0 0 1 0 1 1 1 ? 0 1 0 0 ? 0 1 0 0 1 1 2 0 0

Polymerurus 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0
1 0 0 0 1 0 0 1 0 1 0 0 0 1 1 1 1 1 1 0 1 ? 0 0 1 0 1 1 1 1 0

Proichthydiodes 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 1 0 0 0 1 0 0 1 0 0 0 0 0 1 1 0 ? 1 ? 0 0 ? 1 0 0 0 1 1 2 0 0

Proichthydium 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0
0 1 0 0 0 1 0 0 1 0 0 0 0 0 1 1 0 ? 1 ? 0 0 ? 1 0 0 0 1 1 2 0 0

Rhomballichthys 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0
0 0 1 0 1 0 1 0 0 1 0 1 1 0 0 1 1 1 ? 1 1 1 0 ? 0 0 1 0 1 1 1 1 0

Stylochaeta 1 0 0 1 0 ? 1 0 0 0 0 1 ? 0 0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 1
0 0 0 1 1 0 1 0 0 0 1 1 1 1 0 ? 1 1 0 0 ? 0 1 0 0 1 1 2 0 0

Xenotrichula 1 0 0 1 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 2 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0
? 1 0 1 0 0 0 1 1 0 1 0 0 1 1 1 1 1 1 0 0 1 0 0 1 1 1

# CHAPTER 2

## GASTROTRICHA MACRODASYIDA: A CLADISTIC ANALYSIS OF GASTROTRICH MORPHOLOGY[1]

### Introduction

Gastrotrichs are free-living, microscopic invertebrates with a worldwide distribution in freshwater, estuarine, and marine benthic habitats. Most gastrotrichs are less than 1 mm long as adults and move predominantly by ciliary action. Marine forms inhabit sediment interstices of coastal beaches and continental shelves, while freshwater gastrotrichs lead an epiphytic or semiplanktonic existence (Hummon 1982). Gastrotrichs are common members of the meiobenthos and are often found in association with other microscopic fauna, such as nematodes, turbellarians, and harpacticoid copepods. In intertidal habitats, marine gastrotrichs are occasionally one of the numerically most abundant groups of meiofauna (Hochberg 1999).

The 61 genera and 525 described species of Gastrotricha are divided between the two orders Chaetonotida and Macrodasyida. The principal criteria for distinguishing the orders include general body shape, distribution of adhesive tubules, orientation of the pharyngeal lumen, and the presence of pharyngeal pores (Ruppert 1991). Chaetonotidans

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1. Hochberg, R. & Litvaitis, M.K. 2001c. Macrodasyida (Gastrotricha): a cladistic analysis of morphology. Invertebrate Biology 120: 124-135.

are distributed equally among freshwater and marine habitats, generally have a ten-pin body shape, are hermaphroditic and/or parthenogenic, most with an adhesive tube at each tip of a caudal furca. Macrodasyidans are predominantly marine, hermaphroditic worms with a more elongate body and a complex distribution of adhesive tubes.

While far from speciose, the Gastrotricha show remarkable morphological diversity on a simple vermiform body plan. Ultrastructural studies have contributed a wealth of information on the complexities of body wall structure (Teuchert 1974, 1977; Rieger 1976; Rieger & Rieger 1977; Tyler & Rieger 1980; Tyler et al. 1980; Travis 1983), reproductive anatomy (Teuchert 1976a, 1977; Ruppert & Shaw 1977; Ruppert 1978a,b; Fischer 1996), and nervous system organization (Teuchert 1976b, 1977; Gagne 1980; Wiedermann 1995). Autapomorphies of Gastrotricha initially discovered by light microscopy (Remane 1933, 1936), and later confirmed by electron microscopy, include a bilayered cuticle with basal fibrous/striated zone and outer lamellar zone of multiple membranes (Rieger & Rieger 1977), cuticle-covered locomotory and sensory cilia (Rieger 1976; Rieger & Rieger 1977), and tubular duo-gland adhesive organs (Tyler & Rieger 1980). Other potential autapomorphies, though less certain, include an aberrant form of embryonic cleavage (Sacks 1955; Teuchert 1968) generally similar to that of enoplid nematodes (Malakhov 1994), and the structure of the cerebral ganglion (Wiedermann 1995), similar to that of Cycloneuralia (Schmidt-Rhaesa 1996).

Despite its undoubted monophyly, Gastrotricha has ambiguous sister-group relationships within the Bilateria, sharing a host of morphological characters with Gnathostomulida (monociliated epidermis, Rieger & Rieger 1977; protonephridia structure, Neuhaus 1987; Fischer 1994), Nematoda (bilayered cuticle, Rieger & Rieger

1977; myoepithelial pharynx and nervous innervation, Ruppert 1982), Cycloneuralia (tripartite cerebral ganglion, *sensu* Nielsen 1995), and coelomate Protostomia (circumenteric nerve ring with several ventral nerve cords, *sensu* Brusca & Brusca 1990). Current morphological and molecular analyses have not permitted a consensus on interphyletic relations of the Gastrotricha.

This may, in part, be due to a general lack of understanding of their intraphyletic genealogy and ground pattern organization. Few morphologists have attempted to reconstruct relationships within the phylum (Ruppert 1982; Travis 1983) or to reconstruct its ground patterns (reproductive system of specific families, Ruppert 1978a,b; myoepithelial pharynx, Ruppert 1982; muscle, Travis 1983; protonephridia, Neuhaus 1987). A single molecular study using 18S rRNA sequences found macrodasyidans clustered among a paraphyletic Chaetonotida (Wirz et al. 1999). To date, only one computer-assisted cladistic analysis has been performed using morphological characters (Hochberg & Litvaitis 2000a), confirming monophyly of the phylum, orders, and many families.

Currently, 7 recognized families constitute the order Macrodasyida —Dactylopodolidae, Lepidodasyidae, Macrodasyidae, Planodasyidae, Thaumastodermatidae, and Turbanellidae. Evolutionary relationships of most families are poorly known because of insufficient morphological descriptions and a lack of ultrastructural work on basal taxa. Taxonomic guides have made it relatively simple to place gastrotrichs into their respective genera (Boaden 1963; Hummon 1982; Ruppert 1988), but familial taxonomy is necessarily bypassed because of the apparent lack of well-defined apomorphies. In fact, the paraphyly of Lepidodasyidae (Ruppert 1982,

1991; Travis 1983) has confounded understanding of macrodasyidan relationships, and many genera are phyletically dispersed throughout the orders. Meanwhile, we can only use those data already available to try and assist in the systematic placement of taxa.

The goal of this analysis was a preliminary phylogenetic hypothesis about relationships within the Macrodasyida. Monophyly of the order was tested, and an attempt was made to reconstruct its ground pattern and to examine in-group relationships. I also re-evaluated characters used in previous phylogenetic analyses of Gastrotricha (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000c) and formulated hypotheses about evolutionary character transformations within the order.

## Methods

Cladograms computed in this study are rooted by different outgroups. Macrodasyida is rooted using Gnathostomulida, *Neodasys* (Gastrotricha, Chaetonotida, Multitubulatina), and *Chaetonotus* (Gastrotricha, Chaetonotida, Paucitubulatina). Gnathostomulida and *Neodasys* were chosen as outgroups because they have figured prominently in previous phylogenetic discussions featuring macrodasyidan gastrotrichs (Rieger & Rieger 1977; Ruppert 1980; Tyler et al. 1980). *Chaetonotus* was chosen as the third outgroup based on the results of a previous molecular study of all gastrotrichs (Wirz et al. 1999). A morphological data set consisting of 33 characters and 33 taxa were used for Macrodasyida (Tables 2.1, 2.2). Characters were obtained from literature and direct observations. All characters were left unordered (no specific transformation sequence) and of equal weight.

Maximum parsimony (MP) trees were constructed using the heuristic search option (100 random replicates, tree bisection-reconnection branch-swapping algorithm

with collapsing zero-branch length option; PAUP* v4.0b2a; Swofford 1999). Clade support was estimated by 1000 "full heuristic" bootstrap replications. The limitations of bootstrapping are reviewed in Kitching et al. (1998). Character transformations and reconstructed ancestral states were estimated under the accelerated and delayed transformation optimization (ACCTRAN & DELTRAN, PAUP* v4.0b2a; Swofford 1999). Additional searches were performed using the Branch and Bound search option (furthest addition) and Heuristic Search option (Random search with 2 trees held per step) to check if additional searches would find more MP trees. Agreement subtrees were performed to find clades and taxa common to all MP trees.

Because the MP analyses resulted in some paraphyletic or polyphyletic clades (Lepidodasyidae, Planodasyidae), I developed alternative hypotheses by individually constraining the three families into monophyletic taxa. Constraint trees were compared with MP trees, using the non-parametric, ranked-sign test of Templeton at $\alpha$=0.05 (Larson 1994).

## Results

Parsimony analysis (Branch and Bound, Heuristic Search) resulted in 323 MP trees of 56 steps each (CI=0.607, RI=0.831). Strongly supported taxa included the Dactylopodolidae, Macrodasyidae, and Thaumastodermatidae; the subfamilies Diplodasyinae and Thaumastodermatinae also were supported. Although Turbanellidae (*sensu* Hummon 1974) was supported by fewer than 50% of bootstrap replicates, the clade appeared monophyletic in the consensus cladogram and all 323 MP trees. Monophyly of Lepidodasyidae and Planodasyidae was not supported. When

constraining them into monophyletic groups, the resulting trees were not significantly different from the MP trees (56 and 59 steps, respectively).

Table 2.1. Morphological characters used for phylogenetic analysis. Most characters were scored as absent (a) or present (p). For all other characters, scoring for the matrix in Table 2.2 is given.

**Digestive tract**
1. Myoepithelial pharynx with inverted Y-shaped lumen (a/p)
2. Pharyngeal pores (a/p)
3. Ciliated pharyngeal epithelium (a/p)
4. Ciliated gut epithelium (a/p)
5. Wide buccal region > 50% head width (a/p)

**Body wall**
6. Epidermal cilia (0 = monociliated, 1= multiciliated)
7. Distinct head enclosing pharynx (a/p)
8. Epidermal glands with banded contents (a/p)
9. Scales form solid 'basal' endocuticular thickenings (a/p)
10. Cuticular spines present as triancres, tetrancres, or pentancres (a/p)
11. Hollow scales with epidermal evagination (a/p)
12. Circular muscles in lateral body region (a/p)
13. Y-cells with myofilaments (a/p)
14. Muscular chordoid organ (a/p)
15. Muscle striation (0 = cross-striated, 1 = obliquely-striated)
16. Z-disc organization (0 = dense bodies, 1 = rods)

**Sensory organs**
17. Spatulate-shaped ventrolateral pestle organs (a/p)
18. Dorsal trunk cirrata (a/p)
19. Short cephalic papillae (a/p)
20. Lateral 'segmented' cephalic tentacles (a/p)

**Adhesive tubes**
21. Anterior adhesive tubes (0 = absent, 1 = arc/transverse orientation at mouth rim, 2 = clumped posterior to mouth)
22. Extraordinary "Seitenfüsschen" adhesive tubes (a/p)
23. Round caudal end with adhesive tubes (a/p)
24. Caudal end with a tail bearing adhesive tubes (a/p)
25. Biramous peduncle with proximal/distal separation of caudal adhesive tubes (a/p)

**Reproductive system**
26. Testes (0 = paired, 1 = single right testis only)
27. Anteriorly reflexed vasa deferentia (a/p)
28. Vasa deferentia connect to male caudal organ (a/p)
29. Rosette organ (a/p)
30. Striated cylindrical sheath surrounding sperm flagellar axoneme (a/p)
31. Direction of oocyte maturation (0 = posterior to anterior, 1 = anterior to posterior)
32. Eversible copulatory organ (a/p)
33. Ovaries (posterior to testes) in anterior trunk region (a/p)

Table 2.2. Matrix of 33 characters used in this analysis. Character numbers correspond to the list of characters in Table 1. For characters scored as absent or present: 0 = absent, 1 = present. For polymorphic characters (6, 15, 16, 21, 26 & 31), see Table 1. ? = unknown.

| Taxon | Characters |
|---|---|
| Gnathostomulida | 000000000001000000000000000000?100 |
| *Chaetonotus* Ehrenberg 1830 | 000001000000001100000000000000000? |
| *Neodasys* Remane 1927 | 000000000000000010000200000000000 |
| *Acanthodasys* Remane1927 | 1100?10010001011000010000001111000 |
| *Cephalodasys* Remane 1926 | 1100000000010011000020100000001101 |
| *Chordodasys* Schoepfer-Sterrer 1969 | 1111001100110100000120000000?000 |
| *Crasiella* Clausen 1966 | 1100000000010011000020000000?0?1 |
| *Dactylopodola* Strand 1929 | 11?000100001000000002000000000000 |
| *Dendrodasys* Wilke 1954 | 1111001000010000000020001000?0?0 |
| *Dendropodola* Hummon et al. | 1111?001000010000000020001000?0?0 |
| *Desmodasys* Clausen 1965 | 110?0101000100110000200000100?0?0 |
| *Dinodasys* Remane 1927 | 110?0?01000?00??00012?0000100?0?0 |
| *Diplodasys* Remane 1927 | 1100110010000011000010000001110?0 |
| *Dolichodasys* Gagne 1976 | 1100010000001001100002010000000101 |
| *Hemidasys* Claparède 1867 | 1100?100100000??000010000101O?010 |
| *Lepidodasys* Remane 1926 | 1000010010001011000010100000?0?0 |
| *Macrodasys* Remane 1924 | 1100000000001001100001001000001010 |
| *Megadasys* Schmidt 1974 | 1100000000001001100001010000000?0?0 |
| *Mesodasys* Remane 1951 | 1100000000001001100001010000101000 |
| *Paradasys* Remane 1934 | 11000?00000100??000020100000?1?1 |
| *Paraturbanella* Remane 1927 | 1100010100001001100002100001000000 |
| *Planodasys* Rao 1970 | 11000?00000100110000200000000?0?1 |
| *Platydasys* Remane 1927 | 1100?1000000001100001000010101010 |
| *Pleurodasys* Remane 1927 | 1100010000?00??000021000000?0?0 |
| *Prostobuccantia* Evans & Hummon 1991 | 11000?01000?00??000021000100?000 |
| *Pseudostomella* Swedmark 1956 | 1100110011000011001010000101010?0 |
| *Pseudoturbanella* d'Hondt 196 | 11000?0?000?00??000021000100?000 |
| *Ptychostomella* Remane 1926 | 1100110000000011000010000101010?0 |
| *Tetranchyroderma* Remane 1926 | 1100110011000011111010000101010?0 |
| *Thaumastoderma* Remane 1926 | 1100010011000011111010000101010?0 |
| *Turbanella* Schulze 1853 | 1100010100010011000021000010000000 |
| *Urodasys* Remane 1926 | 1100000000001001100001001000000?010 |
| *Xenodasys* Swedmark 1967 | 1111001100110?000001200000000?000 |

An agreement subtree was calculated to show which clades and taxa were common to all 323 MP trees (Fig. 2.2). Several families remained monophyletic, but within the Lepidodasyidae and Planodasyidae several genera with variable placement were pruned from the tree. ACCTRAN and DELTRAN optimizations were used to estimate character state transformations and to reconstruct ancestral states. For ACCTRAN and DELTRAN optimizations, a single MP tree was arbitrarily selected from among the 323 MP trees and specific character state changes were mapped onto it (ACCTRAN and DELTRAN trees with identical topologies, Fig. 2.3). In addition, ancestral states were reconstructed for taxa found to be monophyletic (Table 2.3).

Based on ACCTRAN and DELTRAN optimizations, the macrodasyidan ground plan was established (Table 2.3). Characters defining the order include an inverted Y-shaped pharyngeal lumen, pharyngeal pores, and anterior adhesive tubes clumped posterior to the mouth (Fig. 2.3). Plesiomorphic features of the Macrodasyida that may constitute the ground pattern for the phylum include a monociliated epidermis, cross-striated muscles, Z-discs organized as an array of dense bodies, circular muscles in lateral body region, paired testes, and paired ova that mature in a posterior to anterior direction (Fig. 2.3, Table 2.3).

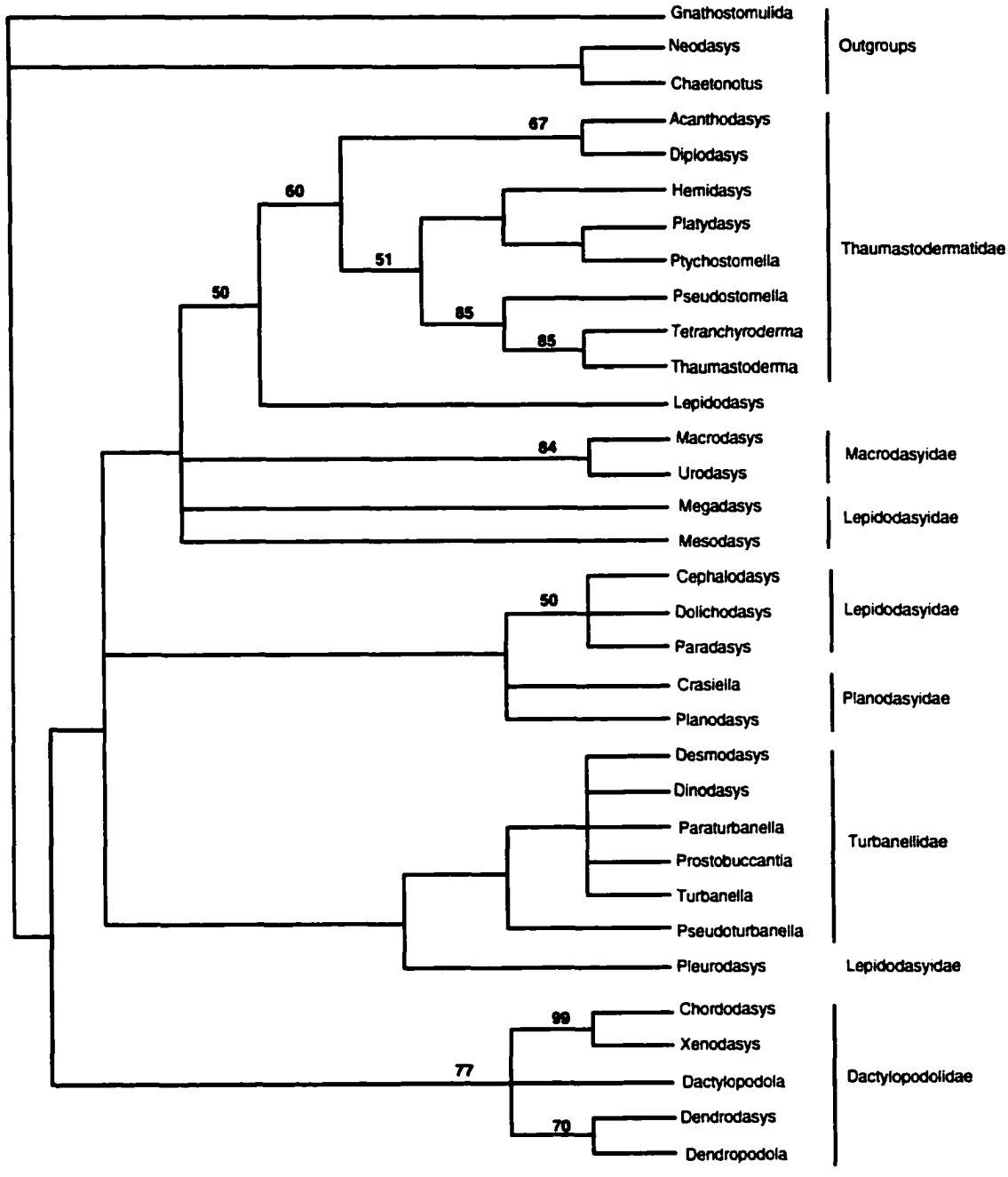


Figure 2.1. Strict consensus tree of gastrotrich morphology. All ingroup taxa are genera from the Macrodasyida. Clades supported by bootstrap values higher than 50% are indicated by numbers above branches.

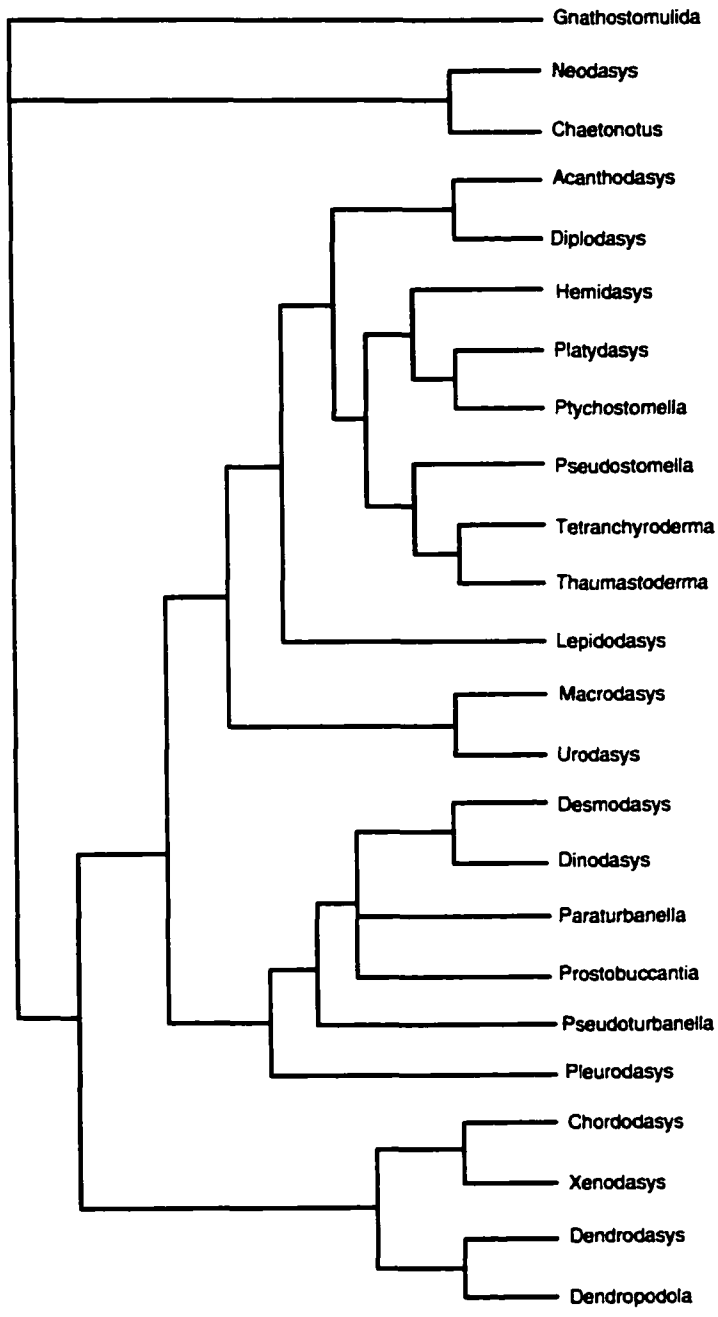


Figure 2.2. Agreement subtree based on taxa and clades that did not change among 323 MP trees.

Table 2.3. Reconstructed ancestral states for the order Macrodasyida and all formally recognized monophyletic taxa based on ACCTRAN optimization. Character states and numbers correspond to those found in Tables 2.1 and 2.2.

| Taxon | Character |
|---|---|
| Order Macrodasyida | 11000000000100000000020000000000000 |
| Family Dactylopodolidae | 11100000000100000000020000000000000 |
| Family Macrodasyidae | 11000000000100110000100100000101O |
| Family Thaumastodermatidae | 11001100100000110000100000010100O |
| Subfamily Diplodasyinae | 11001100100000110000100000011100O |
| Subfamily Thaumastodermatinae | 11001100110000110000100001010100O |
| Family Turbanellidae | 11000101000100110000210000100000O |

## Discussion

### Order Macrodasyida

The order Macrodasyida is well defined in terms of overall morphology and reproductive anatomy, and is usually considered a monophyletic group (Ruppert 1982, 1991; Travis 1983). The analysis clearly supported monophyly (Fig. 2.1). Traditionally, the presence and distribution of adhesive tubules, orientation of the pharynx, and anatomy of the reproductive system were used as a basis for systematics and phylogenetic reconstruction (Remane 1936). We found all of these characters to be valid autapomorphies defining the order (Fig. 2.3, Table 2.3). Additional characters can be found in Table 2.3. Since the comparative ultrastructural studies of Rieger (1976), Rieger

& Rieger (1977), Ruppert (1978a,b, 1982, 1991), and Travis (1983), new morphological data have allowed a re-evaluation of the phylogenetic significance of some characters and a revision of evolutionary hypotheses of the order.

In accordance with other studies, this analysis strongly supported a division of Macrodasyida into at least 4 monophyletic families (Fig. 2.1). Ruppert (1982) presented the first phylogenetic tree of the Gastrotricha based principally on ultrastructural aspects of the myoepithelial pharynx. Travis (1983) followed with a study of body-wall ultrastructure and Y-cell systems. Hochberg & Litvaitis (2000c) attempted a cladistic analysis of the phylum using 81 morphological characters. All three phylogenetic hypotheses agree with the present study in recognizing 4 distinct, monophyletic families—Dactylopodolidae, Macrodasyidae, Thaumastodermatidae, Turbanellidae. Two unnatural groupings (Lepidodasyidae, Planodasyidae) were found in this study and by Ruppert (1982) and Travis (1983). Many of the characters used in the previous analyses (muscle striation patterns, ultrastructure of cells and cell junctions, number of pharyngeal nerves, reproductive organs), while undoubtedly containing important phylogenetic information, remain unknown for many macrodasyidan genera (see Hochberg & Litvaitis 2000c), and, therefore, were of limited value in the current analysis.

Family Dactylopodolidae. The current study resolved Dactylopodolidae as the most basal lineage among Macrodasyida (Fig. 2.1). Other authors also have regarded Dactylopodolidae as the most basal family within the order, i.e., the sister group to all other Macrodasyida (Ruppert 1982; Travis 1983). Characters that substantiate the family's basal position include characteristics of the musculature (#15 & #16) and a monociliated epidermis (#6, unconfirmed in *Dendropodola*). However, the extent to

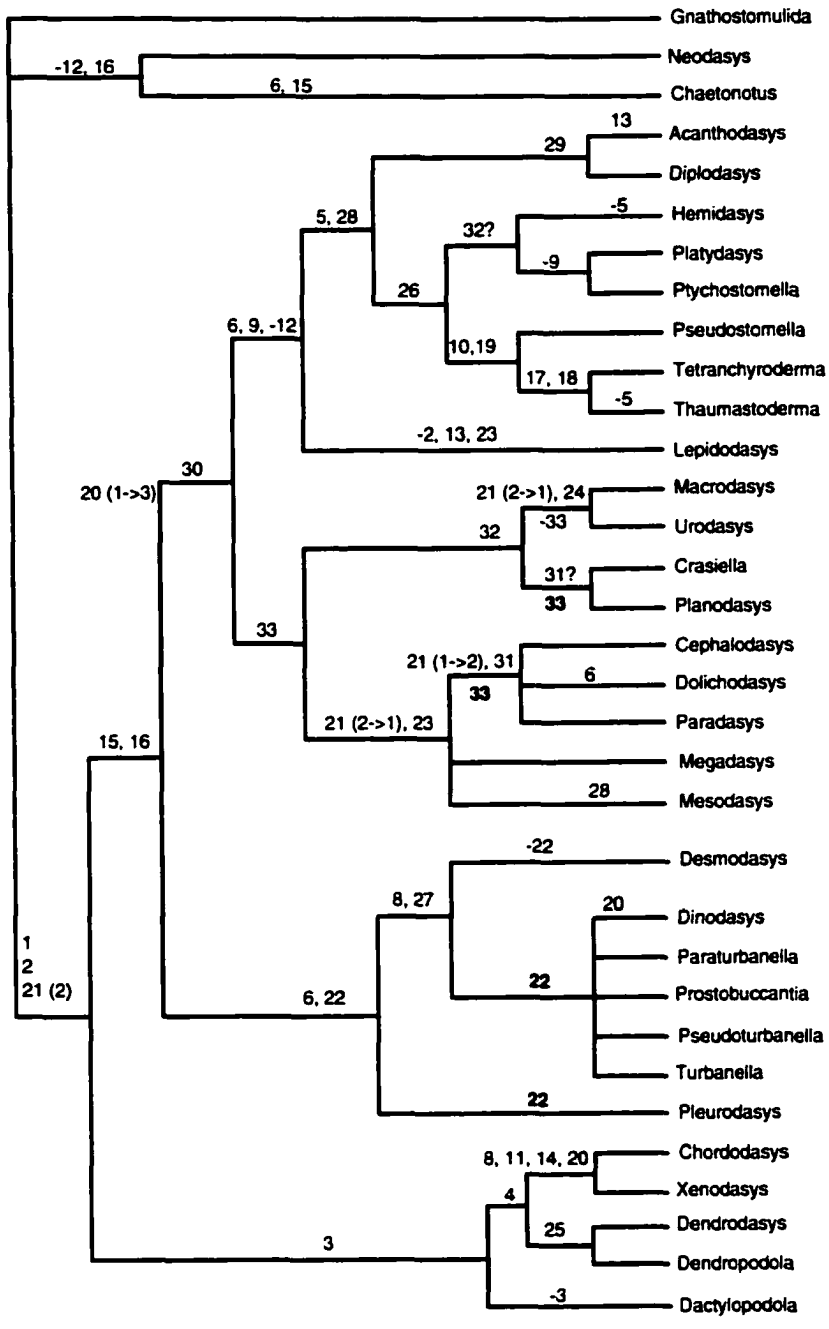


Figure 2.3. Character state transformations according to a single ACCTRAN optimization of a MP tree. Characters are listed at nodes and correspond to those in Table 1. Loss of a character is indicated by "-" and changes in polymorphic character states are indicated in parentheses. Differences in the placement of 2 characters according to DELTRAN are indicated in bold.

which these characters exist in other taxa remains questionable (Table 2.2). Other characters that argue for the basal position of this family include a smooth cuticle and multiple unfused nerve cords (Hochberg & Litvaitis 2000c).

The sister group relationship of *Xenodasys* and *Chordodasys* is confirmed. However, the position of these taxa in the family is contentious (see Hummon 1974; Rieger et al. 1974) since the main characters unifying the Dactylopodolidae are recognized as symplesiomorphies (monociliated epidermis, muscle structure), and the only synapomorphy, ciliated pharyngeal epithelium, requires further examination. Additional morphological research on the family is warranted before accepting the systematic position of *Chordodasys* and *Xenodasys* (see Hummon 1974 for systematic review). We suggest that until such time, these genera should be recognized as divergent members of the Dactylopodolidae.

Family Turbanellidae. The Turbanellidae is one of the most ubiquitous and ecologically diverse macrodasyidan families, traits that suggest an old evolutionary origin. Three synapomorphies support monophyly of Turbanellidae: a multiciliated epidermis (#6), anteriorly reflexed vasa deferentia (#27), and the presence of epidermal glands with banded contents (#8). A multiciliated epidermis appears to have been derived independently at least twice in the Macrodasyida (Rieger 1976). The reflexed vasa deferentia is characteristic of all Turbanellidae. *Paraturbanella,* a genus once thought to lack reflexed vasa deferentia (Ruppert 1988), more recently was found to possess it (*P. aggregotubulata* Evans,1992; W.D. Hummon, pers. comm.). This suggests that the orientation of the vasa deferentia may be highly polymorphic within the Turbanellidae

and within other families (e.g., reflexed vasa deferentia in *Urodasys roscoffensis* Kisielewski, 1987b).

The third autapomorphy, epidermal glands with banded contents, is a more dubious character because it is not shared by all members of the family nor by all species within a particular genus (e.g., *Turbanella*). Further, the ultrastructure of the bands has been described only in a member of another family (*Chordodasys*, Rieger et al. 1974). We interpret the presence of these band-like contents in the glands of *Chordodasys* and the Turbanellidae as convergent (Fig. 2.3) until further information is available.

Lastly, an extraordinary group of ventrolateral adhesive tubes, often referred to as Seitenfüsschen, may form a fourth autapomorphy of the family. However, their presence in all genera has not been confirmed, nor has their structure (single tubes vs. paired tubes). In addition, similar adhesive organs have been found in members of other families (*Pleurodasys megasoma* Boaden, 1963 and *Macrodasys remanei* Boaden,1963) further clouding the situation.

Family Thaumastodermatidae. One of the largest and morphologically most diverse macrodasyidan families, the Thaumastodermatidae, is also well resolved in the analysis (Fig. 2.1). Monophyly is supported by two autapomorphies: sperm ducts that internally connect to the caudal organ (#28, convergent with *Mesodasys*) and a wide flaring buccal cavity (#5). A third autapomorphy is the loss of circular muscles from the lateral body region (#12, Fig. 2.4). This character is shared with *Lepidodasys* (reviewed below) and is part of Ruppert's (1978b) emmended diagnosis of the family.

Within the Thaumastodermatidae, Ruppert (1978b) defined the subfamilies Diplodasyinae (*Acanthodasys, Diplodasys*) and Thaumastodermatinae (*Hemidasys,*

*Platydasys, Pseudostomella, Ptychostomella, Tetranchyroderma, Thaumastoderma*). The analysis resolved the two subfamilies as monophyletic taxa (Fig. 2.1). Diplodasyinae is characterized by loss of the female accessory organ (frontal organ) and subsequent evolution of the rosette organ. According to Ruppert (1978b), losses and gains of these organs are unlikely to be convergent.


Figure 2.4. Fluorescent micrographs showing intestinal and body-wall muscles of gastrotrichs. (**A**) Ventral view of the anterior end of *Tetranchyroderma papii* Gerlach, 1953. Note the presence of circular muscles around the intestine but missing in the body wall, different from that of (**B**) *Dolichodasys elongatus* Gagne, 1977, view of the ventral trunk region. Staining methods for muscle preparation, see Hooge & Tyler (1999). Scale bar, 40 μm.

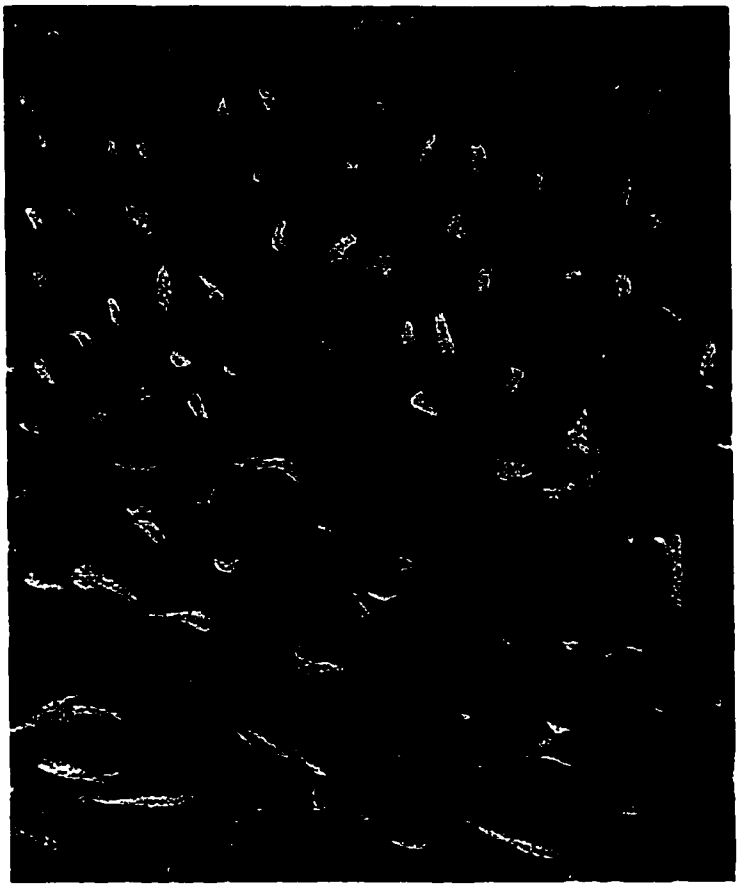

Figure 2.5. Pentancres (#10) of *T. papii.* Scale bar = 10 μm. SEM methods follow Hochberg & Litvaitis 2000b.

The subfamily Thaumastodermatinae is defined by a single autapomorphy, the loss of the left testis (#26). This character is nearly universal for the subfamily, but has occurred sporadically in other taxa, including species of *Urodasys* (Schoepfer-Sterrer 1974). Also, the cuticle has been extensively modified within the subfamily. One well-defined subclade (*Pseudostomella, Tetranchyroderma, Thaumastoderma*) shows elaboration of cuticular hooks into 3-, 4-, and 5-pronged spines (#10, Fig. 2.5), presumably from an ancestral single-spined bowl-shaped scale (Rieger & Rieger 1977).

The other clade (*Hemidasys, Platydasys, Ptychostomella*) has a smooth cuticle, presumably derived through loss of the bowl-shaped scales (Rieger & Rieger 1977). Family Macrodasyidae. Macrodasyidae consists of two genera, *Macrodasys* and *Urodasys*. Both genera contain animals with a thin, elongate tail often bearing numerous adhesive tubules. This unique tail morphology constitutes the single strongest autapomorphy (#24) of the family. A second potential autapomorphy may be found in the structure of the eversible copulatory organ (#32) of *Macrodasys* and *Urodasys*, respectively, but these must be compared to similar organs in other genera before an assessment can be made (Ruppert 1991).

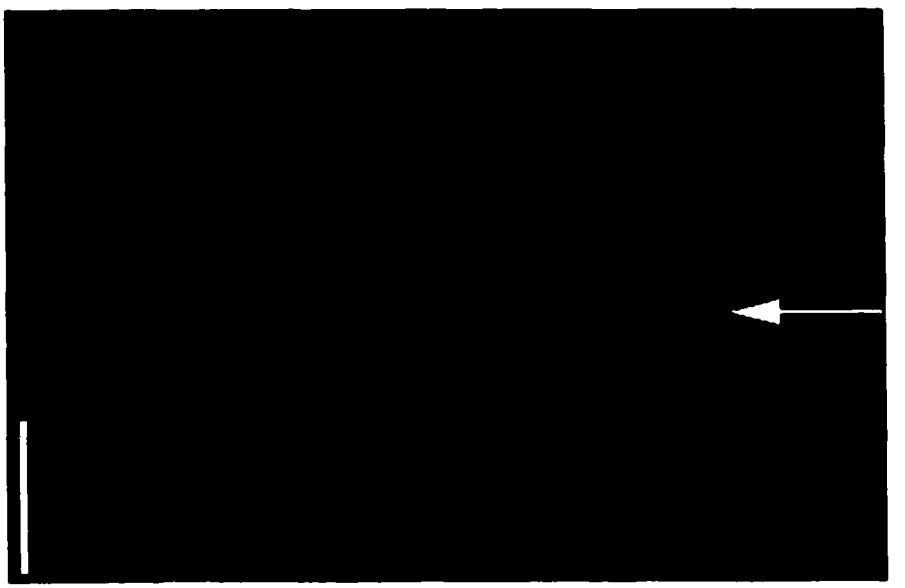

Figure 2.6. Anterior end of *Turbanella mustela* Wieser, 1957 showing adhesive tubes located on hand-like organs (#21, arrow). SEM. Scale bar = 8 μm.

Lepidodasyidae and Planodasyidae. Lepidodasyidae and Planodasyidae remain problematic taxa, without obvious morphological autapomorphies. Neither family forms a monophyletic group (Fig. 2.1), although analyses in which they were constrained as monophyletic clades did not result in significantly longer trees (Lepidodasyidae, 765 trees, 59 steps; Planodasyidae, 322 trees, 56 steps).

Lepidodasyidae is a large heterogeneous group of highly elongate worms (>1 mm). Hummon (1982) and Ruppert (1988) recognized that characters used to define the family (i.e., cephalic pestle organs, cuticle structure, epidermal ciliation, distribution of adhesive tubules, number of ovaries, and direction of ovum maturation) are highly variable and only confuse family definition. The analysis also confirmed Ruppert's (1978b) supposition that *Lepidodasys* is probably an early divergent member of the Thaumastodermatidae or at least shares common ancestry with it (Fig. 3). Synapomorphies that might unite the taxa include a multiciliated epidermis (#6, convergent with Turbanellidae), a similar construction of the cuticle (#9, may be convergent, see Rieger & Rieger 1977), and a loss of circular muscles from the lateral body regions (#12). The presence of a continuous lumen between caudal and frontal organs and the apparent reduction in size of pharyngeal pores within many genera, leading ultimately to their loss in *Lepidodasys,* might be further evidence of close relationships. Additional attention to *Lepidodasys* should clarify its position relative to the Thaumastodermatidae. In the analysis, some lepidodasyid genera were more closely related to each other than to genera of other families (Fig. 2.1). However, they are generally defined on the basis of negative characteristics and warrant further examination (Ruppert 1978a).

Similarly, genera of Planodasyidae (Rao & Clausen 1970) are defined by characters that are either present in other families (#21, diagonal row of anterior adhesive tubules as in *Macrodasys;* #33, anterior location of ova directly behind the testes as in *Dolichodasys*) or are plesiomorphies (i.e., paired ova, biramous caudal end, numerous epidermal glands and lateral adhesive tubules). The only putative autapomorphy for the family appears to be the presence of paired ova in the anterior body region (requiring anterior to posterior maturation as in some Lepidodasyidae, #31). While paired ova are common in Macrodasyida, they usually occur in the posterior body region.

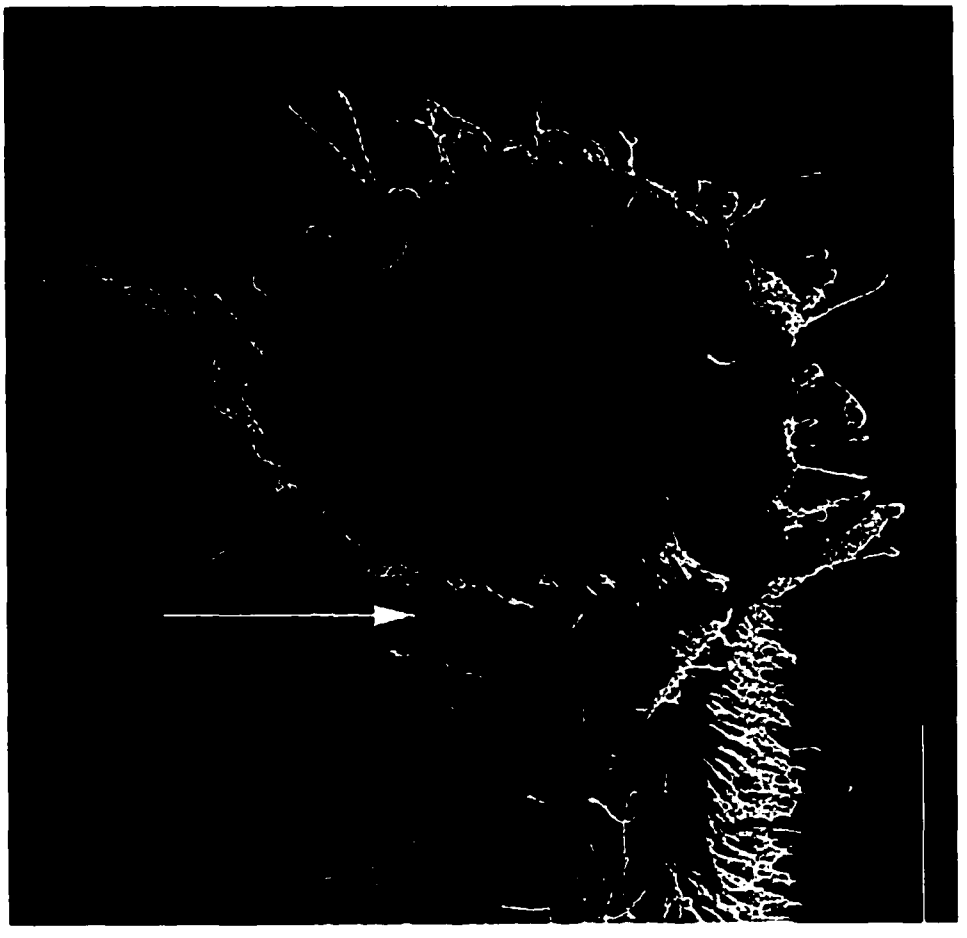

Figure 2.7. Anterior end of *T. papii* showing large buccal cavity (#5) and transverse series of adhesive tubes (#21, arrow). SEM. Scale bar = 15 μm.

Relationships between families

Although we were not able to resolve Lepidodasyidae and Planodasyidae, the analysis identified several macrodasyidan families as valid taxa and we suggest that the more derived taxa may be united by two synapomorphies: muscle ultrastructure and sperm morphology. Ruppert's (1991) ultrastructural analysis of muscle organization in gastrotrichs provides evidence for a change in muscle striation pattern and Z-disc organization between basal and derived clades. All putatively primitive macrodasyidan genera (*Chordodasys*, *Dactylopodola*) have cross-striated muscles with Z-discs organized as an array of dense bodies (similar to Gnathostomulida; Lammert 1991). More derived genera possess obliquely-striated muscles with Z-discs organized as rods. The functional implications of this change in muscle ultrastructure remains to be determined.

The second synapomorphy is a striated sheath that surrounds the 9+2 axoneme of the sperm flagellum (#30) (Balsamo et al. 1999). While this character has been examined in fewer than half the genera, it is so far lacking in Turbanellidae (*Paraturbanella* and *Turbanella*). Two genera from other families, *Dactylopodola* and *Dolichodasys*, have aflagellate sperm. The hypothesis that the striated cylinder represents a synapomorphy of several families (Lepidodasyidae, Planodasyidae, Macrodasyidae, Thaumastodermatidae; Fig. 2.3) is at odds with the hypothesis that it is an autapomorphy of the Macrodasyida (Balsamo et al. 1999). Further observations on other taxa, especially the Planodasyidae and members of the Dactylopodolidae (*Chordodasys* has flagellated sperm; Rieger et al. 1974), are required before either hypothesis can be validated.

The consensus tree provides a working framework for future systematic investigations and affords an opportunity to understand character distribution and transformation series within the order. A single phylogenetic hypothesis (Fig. 2.3) was used to trace character distribution and infer morphological trends. For example, there is a trend in the orientation of anterior adhesive tubes (#20) from complex hand-like structures (some dactylopodolids, some turbanellids, some lepidodasyids) to simple body-wall protuberances (some lepidodasyids, macrodasyids, thaumastodermatids), the selective pressures for which could be sought. Trends within the nervous system are largely unknown, and no nervous characters were included in the present study, because relatively few species have been examined in detail (Rieger et al. 1974; Teuchert 1977; Gagne 1980; Ruppert & Travis 1983; Wiedermann 1995), but some published evidence suggests a tendency towards adnation of nerve cords. For example, in *Dactylopodola baltica* Remane, 1926 there are 4 free nerve cords (Travis 1983); in *Turbanella cornuta* Remane, 1925 4 cords fuse into 2 (Teuchert 1977); and in *Neodasys chaetonotoideus* Remane, 1927 6 cords fuse into 2 (Ruppert & Travis 1983).

Hypotheses of character evolution and of phyletic relationships within the phylum are important to achieve a greater understanding of the ground pattern, which will ultimately help determine relationships within the phylum as well as the phylogenetic position of gastrotrichs among metazoans. We suggest that further research take into account the most basal taxa (Dactylopodolidae, Neodasyidae) as the ones bearing greatest phylogenetic value and thus most likely to provide accurate ground-pattern information.

# CHAPTER 3

## THE MUSCULATURE OF *DACTYLOPODOLA BALTICA* AND OTHER MACRODASYIDAN GASTROTRICHS IN A FUNCTIONAL AND PHYLOGENETIC PERSPECTIVE[1]

### Introduction

Muscle systems of microscopic, soft-bodied invertebrates have recently provided a wealth of new information on development (Rieger *et al.* 1994), functional morphology (Mair *et al.* 1998; Hooge & Tyler 1999a) and systematics (Tyler & Hyra 1998; Hooge & Tyler 1999b). The Gastrotricha comprises but one phylum of exclusively microscopic animals for which muscle systems have provided some insight into the functional aspects of locomotion (Hochberg & Litvaitis 2001b) and phylogeny (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000c, 2001c).

Still, information on the structure and arrangement of muscles in gastrotrichs remains sparse (Remane 1929, 1935-1936; Teuchert 1974, 1977, Teuchert & Lappe 1980; Travis 1983; Ruppert 1991) relative to our knowledge of external morphology (reviewed in Hummon 1982; Ruppert 1991). And while both provide important clues on evolutionary relationships within the phylum, there is a need to find new characters that

1. Hochberg, R. & Litvaitis, M.K. 2001. The musculature of *Dactylopodola baltica* and other macrodasyidan gastrotrichs in a functional and phylogenetic perspecitve. Zoologica Scripta 127: 36-54.

are more conservative across taxa and less prone to phenotypic plasticity (Hummon 1971). Muscle ultrastructure appears conservative at the familial level and displays a correlation with phylogeny (Ruppert 1991), as does overall muscle arrangement (Travis 1983).

From a gross anatomical perspective, the gastrotrich muscular system has received little attention since the monographs of Zelinka (1890) and Remane (1929, 1935-1936). The orientation of specific muscle sets, namely circular and longitudinal muscles, have been more recently analyzed with TEM, providing a sectional view of animals and allowing for inferences on the presence or absence of various muscle groups. This latter information, in conjunction with ultrastructural data on muscle striation patterns and other aspects of the body-wall, has provided a solid foundation for phylogenetic inference (Travis 1983; Hochberg & Litvaitis 200c, 2001c). All such phylogenetic analyses, relying exclusively on morphological data, have shown similar topologies with respect to families in the order Macrodasyida. Interestingly, the family Dactylopodolidae is always shown to be a putative primitive lineage within the Macrodasyida, and a potential sister group to the remaining five families of macrodasyidan gastrotrichs (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 20001c). Noteworthy among the plesiomorphic characters retained within the clade are cross-striated muscles, a monociliated epidermis, and unfused multiple nerve cords. Alone, each of these characteristics may be viewed as secondarily derived from an alternate morphology, but together, they present a strong argument for a basal position of the family based on outgroup analysis (Travis 1983; Hochberg & Litvaitis 20001c).

To the extent that members of the Dactylopodolidae possess primitive structural and organizational features, the topology of the muscular system is likely to reflect a plesiomorphic condition and one central to an understanding of gastrotrich interrelationships. Thus, a comparative study should readily provide some notion of the ancestral status of muscle arrangement and of their principal phylogenetic trends. Here, we describe the muscular system *of Dactylopodola baltica* using a fluorescent F-actin dye to visualize whole-body muscle patterns, and compare these patterns to those present in members of four other families.

## Materials and Methods

The following gastrotrichs were collected from Seabrook Beach, Hampton Harbor, New Hampshire (70° 49' 13" W, 42° 53' 43" N): *Acanthodasys aculeatus* Remane, 1927, *Dactylopodola baltica* Remane, 1926, *Macrodasys caudatus* Remane, 1923, *Pseudostomella roscovita* Swedmark, 1956, *Tetranchyroderma papii* Gerlach, 1953, *Tetranchyroderma megastoma* (Remane, 1927), *Thaumastoderma heideri* Remane, 1926, and *Turbanella ambronensis* Remane, 1943. *Dolichodasys elongatus* Gagne, 1977, was collected from York Beach, Maine (43° 07' 45" N, 70° 37' 27" W) at the mid-tide level (+ 2.0 ft). All gastrotrichs were abundant ($10/cm^2$) in medium to fine grain sand. Additional species from North Stradbroke Island, Queensland, Australia were also collected: *Paraturbanella stradbroki* Hochberg, 2001, *Turbanella brusci* Hochberg, 2002, *Pseudostomella klauseri* Hochberg, 2002 and *P. megalpator* Hochberg, 2002. Gastrotrichs were extracted from the sand using an anesthetization/decantation technique with 7.5% magnesium chloride (Pfannkuche & Thiel, 1988). Gastrotrichs were relaxed in 7.5 % MgCl2 for 10 min prior to a 1 hr fixation in 4% formaldehyde in 0.01M phosphate

buffered saline (PBS). Fixed specimens were rinsed in 0.01M PBS, permeabilized for 1 hr in 0.2% Triton X-100 in PBS, stained for 40 min with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR), and rinsed again in PBS before mounting with Gel/Mount (Biomeda Corp.). New Hampshire specimens were viewed on a Zeiss epifluorescence microscope equipped with Spot Cooled Color digital camera (Diagnostic Instruments, Inc.). Australian specimens were viewed on an Olympus BX60 fluorescence microscope at the University of Queensland, Australia. Measurements of gastrotrichs were performed with an ocular micrometer and the positions of particular organs are expressed in reference to percentage body units (total body length =100 units (U)).

Characteristics of the muscular system were coded as phylogenetic characters. Maximum parsimony (MP) trees were constructed using the heuristic search option (100 random replicates, tree bisection-reconnection branch-swapping algorithm with collapsing zero-branch length option; ACCTRAN optimization; PAUP* v4.0b1a; Swofford 1999). Clade support was estimated by 1000 "full heuristic" bootstrap replications. *Dactylopodola baltica* was used as the outgroup to determine character transformations within the Macrodasyida.

Table 3.1. Characters of the muscular system used in the phylogenetic analysis. Most characters were scored as absent (a) or present (p) unless otherwise noted (#9, #14).

| Character |
|---|
| 1. Somatic circular muscles (a/p) |
| 2. Circular muscles on pharynx (a/p) |
| 3. Thin semicircular muscle band on ventral side of pharynx (a/p) |
| 4. Circular muscles on intestine (a/p) |
| 5. Dorsal longitudinal muscles (a/p) |
| 6. Ventral longitudinal muscles (a/p) |
| 7. Ventrolateral longitudinal muscles (a/p) |
| 8. Bifurcation of ventrolateral longitudinal muscles in trunk region (a/p) |
| 9. Anterior insertion of ventrolateral muscles (0 = behind mouth, 1 = on mouth rim) |
| 10. Cross-over muscles in caudal region (a/p) |
| 11. Myocyte branches from ventrolateral muscles supply head (a/p) |
| 12. Helicoidal muscles on pharynx (a/p) |
| 13. Helicoidal muscles on intestine (a/p) |
| 14. Muscle striation pattern (0 = cross-striated, 1 = oblique striation) |

Table 3.2. Matrix of 14 muscle characters used in this analysis. Character numbers correspond to the list of characters in Table 1. For characters scored as absent or present: 0 = absent, 1 = present. For polymorphic characters (9, 14), see Table 1.? = unknown.

| Taxa | Characters |
|---|---|
| F. Dactylopodolidae | |
| *Dactylopodola baltica* | 1 1 1 1 1 1 1 1 0 1 1 1 1 0 |
| F. Lepidodasyidae | |
| *Dolichodasys elongatus* | 1 1 0 1 1 1 1 1 0 0 0 1 0 1 |
| F. Macrodasyidae | |
| *Macrodasys caudatus* | 1 1 0 1 1 1 1 ? 1 0 0 1 0 1 |
| F. Turbanellidae | |
| *Turbanella ambronensis* | 1 1 1 1 1 1 1 1 0 1 1 1 0 1 |
| F. Thaumastodermatidae | |
| S.F. Diplodasyinae | |
| *Acanthodasys aculeatus* | 0 1 0 1 1 1 1 1 1 0 0 1 1 1 |
| S.F. Thaumastodermatinae | |
| *Pseudostomella roscovita* | 0 1 0 1 1 1 1 0 1 1 0 1 1 1 |
| *Tetranchyroderma papii* | 0 1 0 1 1 1 1 0 1 1 0 1 1 1 |
| *Tetranchyroderma megastoma* | 0 1 0 1 1 1 1 0 1 1 0 1 1 1 |
| *Thaumastoderma heideri* | 0 1 0 1 1 1 1 0 1 1 0 1 1 1 |

## Results

### Musculature of *Dactylopodola baltica*

Circular Muscles. Circular muscles were present as hoop-like rings around the pharynx (Fig. 3.1A) and intestine, and surrounded the ventrolateral muscles of the lateral trunk region. The number of circular rings differed between pharynx and intestine, and varied between specimens of different body lengths. On the pharynx, circular muscles (2 μm wide) were internal to all longitudinal muscles. On the intestine, circular muscles appeared external to most of the longitudinal bands. Somatic circular muscles were also present lateral to the intestine, surrounding the ventrolateral longitudinal muscles.

Longitudinal Muscles. Longitudinal muscles spanned the length of the specimens in dorsal, lateral, ventral and ventrolateral positions. Approximately 6-8 dorsal muscles, each 2 μm wide, inserted anteriorly at the mouth rim and posteriorly on the anus or body mid-line posterior to the anus. A single pair of longitudinal muscles appeared lateral to the digestive tract, but the insertion points could not be determined. At least 4 longitudinal muscles were observed on the ventral side of the pharynx, inserting anteriorly on the mouth rim. Posteriorly, the medial pair of ventral longitudinal muscles (3-4 μm wide) bifurcated at U75 (Fig. 3.2). A total of six ventral longitudinal muscles inserted close to the anus.

The ventrolateral longitudinal muscles were the largest diameter muscles in the body. Each muscle unit appeared to be composed of at least 6-8 individual cross-striated myocytes. Anteriorly around U10, the ventrolateral muscles flared laterally from the pharynx, forming a half-cup shape (muscular cup, Fig. 3.1B, 3.2B), and turning back medially to contact the pharynx. At the sites of lateral flaring and medial contact were

two, ventral, semicircular muscle bands that connected the paired ventrolateral muscles (Fig. 3.1B, 3.2B).

Several muscles appeared to branch off from the ventrolateral bands at their anterior end. A single pair of fine myocytes supplied the dorsolateral portions of the head. Two pairs of thicker muscles also appeared to branch off from the ventrolateral bands and run dorsally over the pharynx (Fig. 3.2A). Two of these muscles crossed each other at approximately mid-pharynx, and appeared to insert on the contralateral side of the pharynx. A second set of muscles, slightly anterior to the first pair and oriented parallel to them, inserted on the diagonal branch of the contralateral muscle.

Posteriorly, each ventrolateral longitudinal muscle bifurcated once around U30-35 and then reunited at U75-80. The precise location of splitting often differed among individuals and, in some cases, the anterior portion of the bifurcation was only evident at high magnification. Around the location of the lateral adhesive tubes, small triangular muscle branches of the ventrolateral bands appeared to be directed towards the adhesive tubes, but did not appear to supply the tubes directly (Fig. 3.2B). At the caudal end, the ventrolateral bands branched three times before insertion into the caudal lobes. The first two branches were directed dorsally, and anastomosed at the body mid-line in two separate locations (adb, pdb, Fig. 3.1C). Posterior to the anus, a single myocyte from each ventrolateral muscle crossed over to the contralateral band at U96 (cross-over muscles, Fig. 3.1D, 3.2B).

Helicoidal Muscles. Helicoidal muscles were arranged in left- and right-handed spirals, beginning at approximately 50% pharynx length and ending close to U75 along the intestine. Where helices crossed, they formed an angle between 50-60° with respect to the

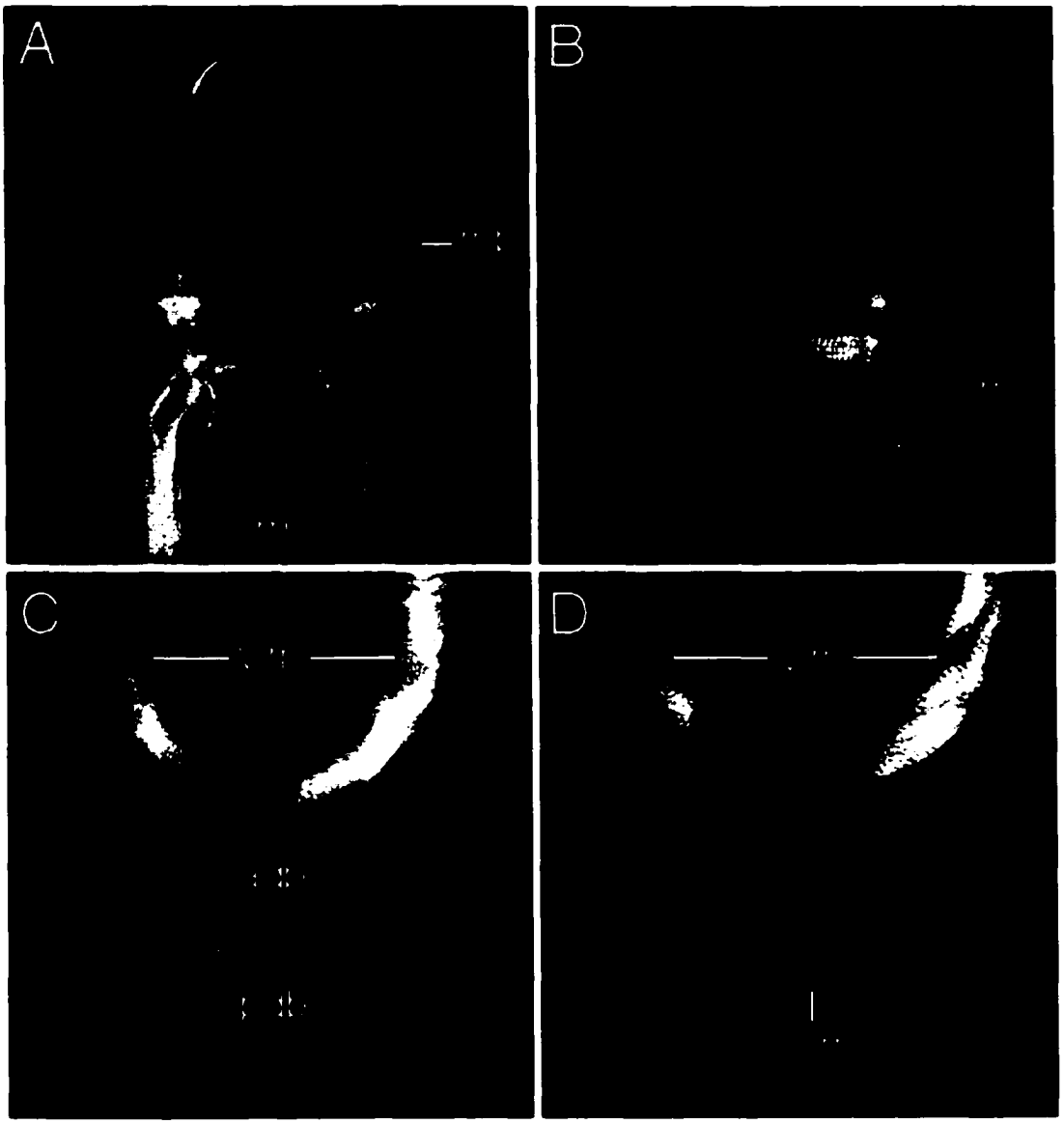


Figure 3.1 A-D. Musculature of *Dactylopodola baltica* stained with Alexa Fluor 488 phalloidin. – A. Dorsal view of anterior end. – B. Ventral view of anterior end. – C. Dorsal view of caudal end. –D. Ventral view of caudal end. adb, anterior dorsal branch of vlm at caudal end; asm, anterior semi-circular muscle; cm, splanchnic circular muscle; com, cross-over muscle; mb, muscle branch of vlm; mc, muscle cup of vlm; pdb, posterior dorsal branch of vlm at caudal end; psm, posterior semicircular muscle; vlm, ventrolateral muscle.

longitudinal axis of the body. Helices were continuous anterior to posterior, looping completely around the digestive tract and associated musculature. In a mature specimen (length = 300 μm), approximately 6 helices were observed on the intestine. Helicoidal muscles did not appear to surround the large ventrolateral bands.

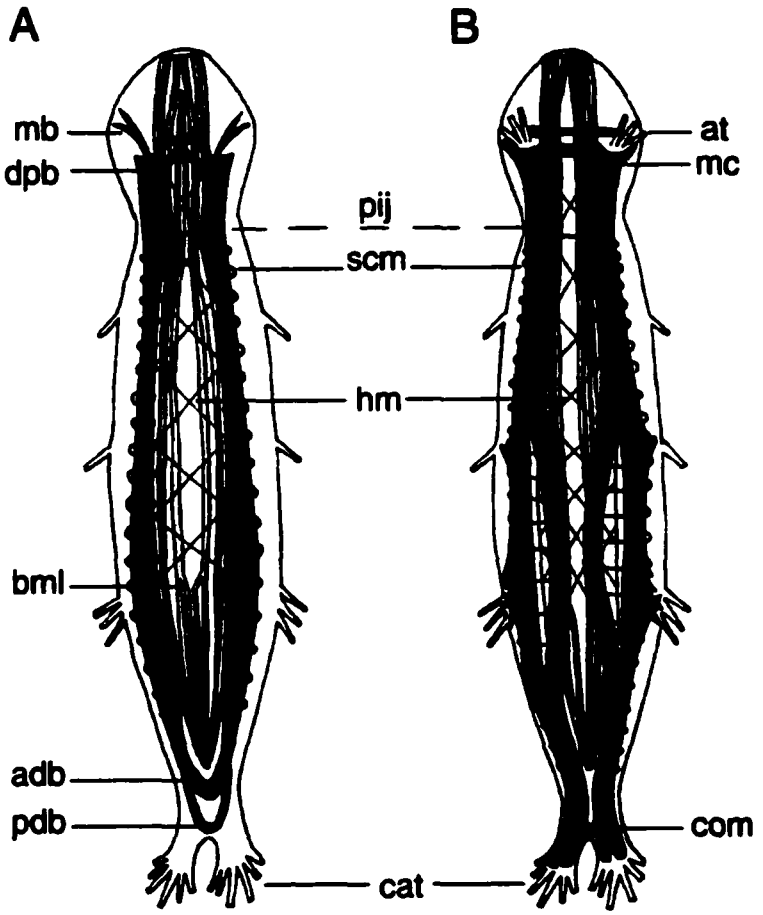


Figure 3.2 A-B. Diagram of body shape and musculature of *Dactylopodola baltica*. Splancnic circular muscles are not illustrated for clarity. - A. Muscles visible in dorsal view. - B. Muscle visible in ventral view. adb, anterior dorsal branch of vlm at caudal end; at, anterior adhesive tubes; bml, bifurcation of medial longitudinal muscle; cat, caudal adhesive tubes; com, cross-over muscle; dpb, muscle branch of vlm on dorsal pharynx; hm, helicoidal muscle; lm, splancnic longitudinal muscle; mb, muscle branch of vlm; mc, muscle cup of vlm; pdb, posterior dorsal branch of vlm at caudal end; pij, pharynx-intestine junction; scm, somatic circular muscle; vlm, ventrolateral muscle.

Musculature of Other Macrodasyida

Circular Muscles. Circular muscles in splanchnic positions (lining the entire digestive tract) and somatic positions (surrounding ventrolateral longitudinal muscles on either side of the intestine) were present in *Dolichodasys elongatus*, *Macrodasys caudatus*, *Paraturbanella stradbroki* and *Turbanella ambronensis*, and *T. brusci*. Members of the Thaumastodermatidae (*Acanthodasys aculeatus*, *Pseudostomella roscovita*, *P. klauserae*, *P. megapalpator*, *Tetranchyroderma megastoma*, *T. papii*, *Thaumastoderma heideri*), had only splanchnic circular muscles (Fig. 3.3A). Some species possessed a distinct sphincter of circular muscles at the mouth rim (Thaumastodermatidae). In *Tetranchyroderma megastoma*, *T. papii*, and *Thaumastoderma heideri*, splanchnic circular muscles extended dorsally beyond the terminal mouth and into the oral hood.

Longitudinal Muscles. Longitudinal muscles showed the most variation in terms of position and orientation. All species possessed thin dorsal and ventral longitudinal muscles lining the pharynx and intestine. Further, in all species except in members of the subfamily Thaumastodermatinae (*Pseudostomella*, *Tetranchyroderma*, *Thaumastoderma*), the anterior insertion for ventral and dorsal longitudinal muscles was the mouth rim. In the Thaumastodermatinae, ventral muscles inserted on the mouth rim (Fig 3.3A), but thin dorsal longitudinal muscles branched several times before insertion into the oral hood (*Tetranchyroderma*, *Thaumastoderma*) or palps (*Pseudostomella*).

Longitudinal muscles lining the intestine appeared to be continutations of muscles from the pharynx, though in species of *Tetranchyroderma*, some muscles appeared to originate from a position on the intestine. The number and diameter of longitudinal muscles varied among all species. All longitudinal muscles, with the exception of the

ventrolateral muscles, were bound by helicoidal muscles (described below). The largest (thickest diameter) muscles in the body were always the paired, bilateral ventrolateral muscles. These muscles were on either side of the digestive tract, often extending from the pharynx to the caudal end. Anteriorly, the ventrolateral muscles inserted either at the position of the ventral adhesive tubes behind the mouth (*Dolichodasys elongatus*, *P. stradbroki*, *Turbanella ambronensis*, *T. brusci*; Fig. 3.3C) or on the pharynx along the mouth rim (*Macrodasys caudatus*, Thaumastodermatidae; Fig 3.3A,B). In the former condition, ventrolateral muscles flared out from the pharynx, probably inserting on the epidermis beneath the cuticle. Posteriorly, in three species (*Acanthodasys aculeatus*, *Turbanella ambronensis*, *T. brusci*), the ventrolateral bands bifurcated posterior to the pharyngeal-intestinal junction, finally reuniting prior to insertion at the caudal end. Ventrolateral muscles inserted either in the caudal lobes *(P. stradbroki*, *T. ambronensis*, *T. brusci*, Thaumastodermatidae), or, alternatively, at the body midline (*D. elongatus*, *M. caudatus*).

Helicoidal Muscles. The number and position of helicoidal crosses varied among taxa. Helicoidal muscles lined the complete digestive tract of members of the Thaumastodermatidae (*Acanthodasys aculeatus*, *Pseudostomella roscovita*, *Thaumastoderma heideri*, *Tetranchyroderma megastoma*, *T. papii*). In *T. heideri* and both species of *Tetranchyroderma*, helicoidal muscles made a small contribution to the oral hood. In *Dolichodasys elongatus*, *Macrodasys caudatus* and species of Turbanellidae, helicoidal muscles were restricted mainly to the region of the pharynx, with a single helix present below the pharyngeal-intestinal junction.

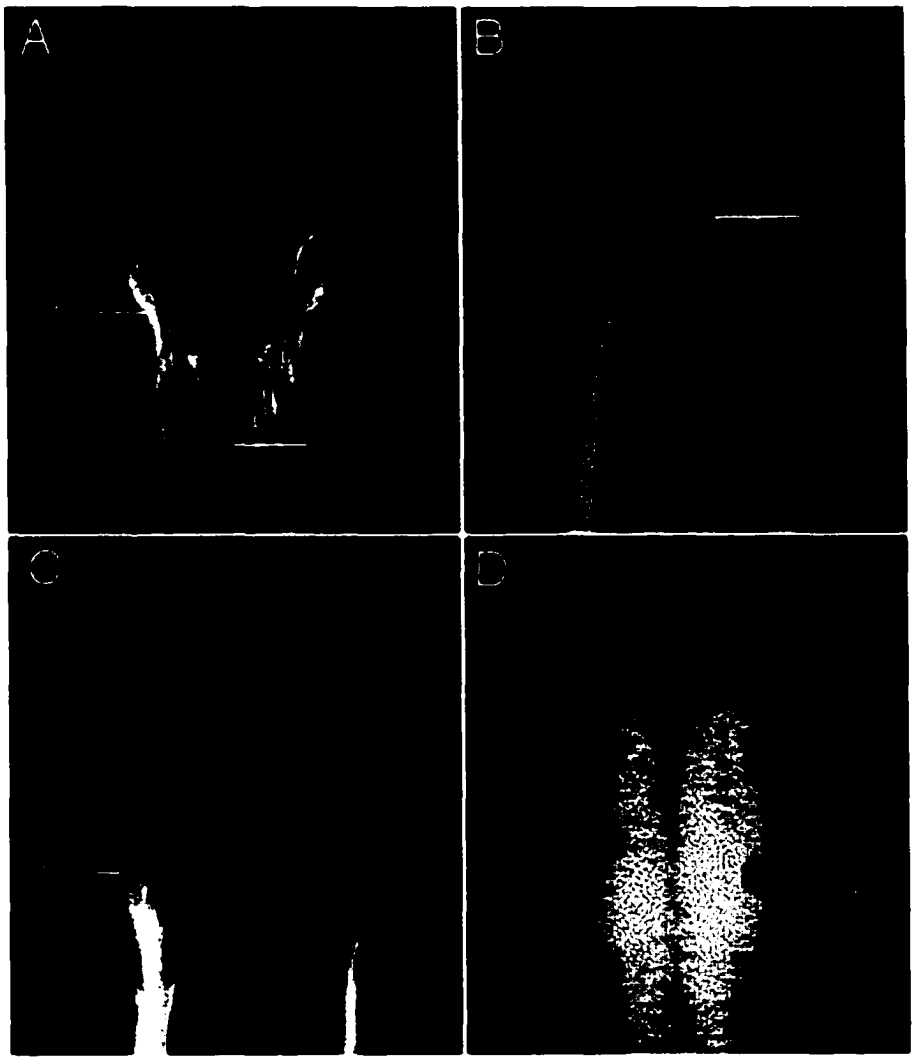


Figure 3.3A-D. Muscles associated with the pharynx of three species of Macrodasyida. – A. *Tetranchyroderma megastoma*, ventral view of pharynx. – B. *Macrodasys caudatus*, dorsal view of pharynx. – C. *Turbanella ambronensis*, ventral view of pharynx. – D. *T. ambronensis*, dorsal view of pharynx. dm, dorsal longitudinal muscle; hm, helicoidal muscle; mb, muscle branch of ventrolateral band; sm, semicircular muscle; vlm, ventrolateral muscle.

Alternate Muscle Arrangements. Several unusual muscle orientations were observed in *Turbanella ambronensis*. Close to the anterior end of the ventrolateral muscles, a single myocyte branched off and supplied the dorsolateral portion of the head (Fig. 3.3D), close to the posterior head cones. Ventrally, a single semi-circular muscle band (1 μm) was present on the pharynx in between the ventrolateral muscle bands (Fig. 3.3C). The semicircular muscle band did not appear to extend to the dorsal side of the pharynx nor connect the ventrolateral muscles.

In the caudal end of several species, diagonal, "cross-over" muscles split off from their respective ventrolateral band and crossed over to the contralateral muscle. Cross-over muscles were noted for three species of *Pseudostomella*, *Thaumastoderma heideri*, *Tetranchyroderma megastoma*, and *T. papii*.

Phylogenetic Analysis

An analysis of fourteen muscle characters observed in this study (Tables 3.1, 3.2) resulted in a single most parsimonious tree (L = 10, CI = 0.800, RI = 0.857; Fig. 3.4). Most nodes were supported by bootstrap values greater than 50%. Muscle characters mapped onto a phylogenetic tree of similar topology (Hochberg & Litvaitis 2000c) showed potential synapomorphies for higher taxa (Fig. 3.5).

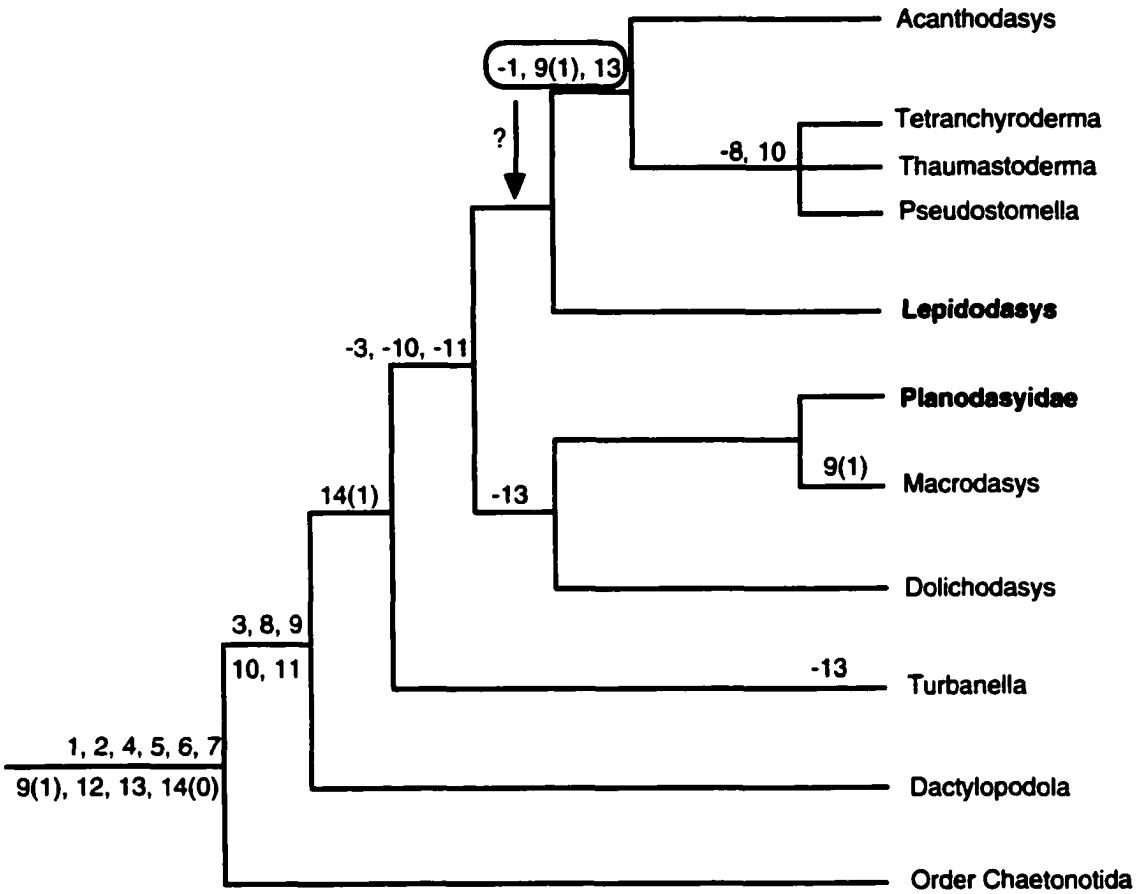


Figure 3.4. Character states mapped onto a modified phylogeny of the Macrodasyida (see Hochberg & Litvaitis 2000c). Terminal nodes of the original tree were collapsed, making genera in this study representative of their respective monophyletic clade. Taxa in bold were not described in this study, but kept in the tree due to their potential systematic importance. Character descriptions are provided in Table 3.1.

## Discussion

### General Arrangement and Function of Gastrotrich Muscles

The arrangement and function of muscles in gastrotrichs is closely related to the hydrostatic nature of their acoelomate organization. Muscles are arranged as a series of bands along the digestive tract and closely aligned with the other organ systems of the body. The musculature of all species examined here is arranged in essentially three orientations: longitudinal, circular, and helicoidal. Several variations, some of which are unique to species and others that may be interpreted as synapomorphies, can be found for each arrangement.

Longitudinal muscles generally span the length of most all gastrotruchs in dorsal, lateral, ventral, and ventrolateral positions, and presumably play a role in general body flexion. Changes in direction during ciliary gliding are initiated by lateral or ventrolateral longitudinal muscles, or, when gastrotrichs remain stationary, these same muscles may initiate backward or froward creeping (Teuchert 1978; Hochberg and Litvaitis 2001b). Mating behavior, with its often complicated twists and turns between individuals (see Teuchert 1968, Ruppert 1978a), probably involves all groups of longitudinal muscles.

Circular muscles generally lie inside of longitudinal bands along the pharynx, whereas the condition is reversed along the intestine (Ruppert 1991). The two arrangements of circular muscles in gastrotrichs, as splanchnic and somatic components, are not independent of each other as they are in other invertebrates (e.g., Polychaeta). Instead, splanchnic circular muscles send out lateral branches to encircle organs on either side of the digestive tract (i.e. somatic component). This branching makes cross-sections of gastrotrichs appear tripartite in organization (three body chambers), with each chamber

surrounded by its own circular muscle component. The circular muscle component of gastrotrichs probably serves a dual-role in antagonizing longitudinal contraction of the body and radial dilations of the pharynx and intestine (Ruppert 1991). The latter function may be aided in part, by helicoidal muscles lining the digestive tract. Together, this muscle pair may also play a role in peristaltic movement of food items (diatoms) down the intestine, since motile, intestinal cilia are lacking from most species (Ruppert 1991). As noted previously (Hochberg & Litvaitis 2000b), the 55° angle of most helicoidal muscles might also be important in preventing kinking of the intestine during severe body contraction or bending. Whole-body contraction is typically fast (see Ruppert & Travis 1983), and while re-extension appears slower in most species, it is generally assumed to be a function of antagonistic muscles. Somatic circular muscles of the lateral body regions are likely to function as the primary antagonists to the large, ventrolateral bands, while splanchnic circular muscles oppose the thinner dorsal and ventral longitudinal muscles. Travis (1983) hypothesized that the absence of somatic circular muscles from Thaumastodermatidae might be functionally correlated with the presence of a sculptured cuticle. Such an complex cuticle may function in elastic recoil more readily than a smooth cuticle, bypassing the need for a muscular antagonist to the ventrolateral bands.

Comparative Muscle Patterns on the Pharynx

The general arrangement of muscles lining the pharynx of *Dactylopodola baltica* is similar to that displayed by the seven other gastrotrich species examined. Circular muscle rings are abundant and surround the pharynx from its tip to the pharyngeal-intestinal junction. In some species, a distinct sphincter is present as closely-set circular muscles around the mouth rim (Thaumastodermatidae). The number of circular muscles

is likely to be a function of ontogeny and perhaps is species dependent. More than 50 splanchnic circular muscles were counted on the pharynx of all species examined (body length > 250 μm).

Numerous longitudinal bands exist outside of the splanchnic circular muscles. In *D. baltica*, approximately 6 longitudinal bands were present ventrally and 8 longitudinal bands present dorsally. Some of these may be a result of muscle branching. The number of longitudinal muscles may also be species-specific. While it was difficult to accurately count the number of longitudinal bands in many species, evidence from ultrastructural observations (Travis 1983) suggest that the quantity of bands might be correlated with phylogeny (discussed below). Interestingly, the number of longitudinal bands visualized in fluorescent micrographs and TEM micrographs (unpublished) of *D. baltica* are approximately the same but different from that seen in illustrations of TEM reconstructions of the same species (Teuchert & Lappe 1980). The cause for this discrepancy is likely to be due to muscle cells abutting each other and appearing as a single unit in TEM micrographs.

Helicoidal muscles, in left- and right-hand spirals, appeared to surround circular and longitudinal muscles of the pharynx. The number of helices differed among species, but all helices formed similar angles (50-60°) with respect to the longitudinal body axis. In all species, helices were present along the entire pharynx. The most anterior helices of *D. baltica* were difficult to visualize and count due to the presence of muscle branches from the ventrolateral bands. In three members of the subfamily Thaumastodermatinae, *Tetranchyroderma megastoma*, *T. papii* and *Thaumastoderma heideri*, helicoidal muscles made a small contribution to the oral hood.

Comparative Muscle Patterns on the Intestine

Circular muscles lined the intestine of all species from the pharyngeal-intestinal junction posterior to the anus. Circular muscle rings were often more widely spaced than those on the pharynx, and appeared to surround thin dorsal and ventral longitudinal bands. All longitudinal muscles in the trunk region are intimately associated with the intestine or organs positioned dorsal (developing eggs) or lateral (gonads) to the intestine. Longitudinal muscles on the intestine appeared to be continuations from the pharynx, but further ultrastructural observations are required to confirm this in all examined species. The number of longitudinal muscles initially appears to be species-specific or perhaps family-specific. The largest number of dorsal longitudinal muscle bands was found in *D. baltica* (6 muscles). In general, fewer dorsal longitudinal bands were present in most other species (3-4 muscles). All longitudinal muscles, except the ventrolateral bands, appeared to insert postanally at the body mid-line or close to the intestine (see Hochberg and Litvaitis 2001b for *Tetranchyroderma papii*).

The largest muscles in all examined species are the ventrolateral longitudinal bands. This muscle pair generally extends the length of an individual, and presumably functions in general body flexion in all species (Hochberg & Litvaitis 2001a). As in most other macrodasyidans, the ventrolateral muscles of *Dactylopodola baltica* have an anterior insertion at the level of the ventral adhesive tubules behind the mouth. However, the precise point of insertion for these muscles differs from all other species examined, because the muscle tips form an unusual muscular cup ventrolateral to the pharynx (Figs. 3.1B, 3.2B). This seemingly complex muscle orientation is a functional anomaly, as the muscle does not appear to form a normal attachment to the cuticle as in *Turbanella* or an

attachment to the pharynx as in *Macrodasys*. An ultrastructural analysis of this muscle orientation and its point of insertion should help to clarify the functional differences between it and arrangements observed in other species. Furthermore, the general site of muscle insertion appears correlated with the presence and location of the anterior adhesive tubes. If this is true across taxa, then we may expect two systematic groupings based on this character: (Dactylopodolidae + Planodasyidae +Turbanellidae + some Lepidodasyidae (*Cephalodasys*, *Dolichodasys*, *Lepidodasys*, *Paradasys*, *Pleurodasys*)) and (Macrodasyidae + Thaumastodermatidae + some Lepidodasyidae (*Megadasys*, *Mesodasys*)).

The caudal arrangement of the ventrolateral muscles also shows some peculiarities among taxa. The caudal site of muscle insertion is generally in one of two positions: within the caudal furca (*Dactylopodola*, *Pseudostomella*, *Tetranchyrodmera*, *Thaumastoderma*) or at the body mid-line in species without a caudal furca (*Dolichodasys*, *Macrodasys*). In species with a forked end, there is often a pair of individual myocytes that cross from the ipsilateral to the contralateral muscle (cross-over muscles). The only examined exceptions are in *Acanthodasys aculeatus* and in *Turbanella ambronensis*. The absence of cross-over muscles from *T. ambronensis* may be species-specific since other members of the family possess them (Remane 1929; Travis 1983). The functions of cross-over muscles remain a matter of speculation, but we hypothesize that they may serve to pull the caudal ends of the ventrolateral bands closer together, thereby buckling the body mid-line and bringing lateral adhesive tubes into contact with the substrate. An additional muscle arrangement seen only in the caudal end of *D. baltica*, present as dorsal branches of the ventrolateral bands (Fig. 3.1C), may also

function in movement of the caudal end. In this case, the likely function is dorsal tail flicking, as observed by Ruppert (1991). Additional attention to the behavior of all these species is likely to provide important clues to the functions of these unusual muscle arrangements.

Evolution and Phylogeny

The comparative analysis of muscle topology has indicated many evolutionary variations among macrodasyidan gastrotrichs. Two approaches were taken to gain insight into the evolution of particular muscle orientations. The first approach involved coding muscle attributes as phylogenetic characters and producing a cladogram. The resulting single most-parsimonious trees displayed a topology consistent with our knowledge of gastrotrich relationships (Fig. 3.4; see Hochberg & Litvaitis 2000c). The second approach involved mapping muscle characters onto an existing cladogram (Fig. 3.5). This approach is slightly problematic because not all taxa in the tree were analyzed in this study. Nevertheless, character mapping provides insight into evolutionary transformations of specific muscle groups and allows for visualization of muscle characters that may form potential synapomorphies for select clades. For example, Macrodasyidae and Thaumastodermatidae are defined by the anterior insertion of the ventrolateral muscles (Fig. 3.5), which may be functionally correlated with the secondary movement of the anterior adhesive tubes to the mouth rim. As indicated in Figure 3.5, the insertion of these muscles may also be convergent. Thaumastodermatidae is further defined by the loss of somatic circular muscles (also known for *Lepidodasys*, Ruppert 1978b; see Fig. 3.5) and subfamily Thaumastodermatinae is characterized by the apparent loss of the ventrolateral muscle division in the trunk region.

As alluded to by Travis (1983), and displayed in Figure 3.5, the musculature of *Dactylopodola baltica* is similar to the musculature expected for the hypothetical ground pattern of the Gastrotricha based on outgroup analysis: splanchnic and somatic circular muscles, dorsal and ventral longitudinal muscles, and ventrolateral muscle bands. Further information from members of the order Chaetonotida (Hochberg & Litvaitis 2001a) suggests that helicoidal muscles lining the intestine are also part of the ground pattern of the Gastrotricha. Helicoidal muscles have apparently been lost or reduced several times throughout the Macrodasyida (*Dolichodasys*, *Paraturbanella*, *Turbanella*, *Macrodasys*). The ground pattern for the Macrodasyida is also defined by additional muscle characters including the anterior insertion of the ventrolateral muscles behind the mouth and at the site of the anterior adhesive tubules, and the posterior splitting of the ventrolateral bands. While the function of the ventrolateral muscle division remains unknown, it is interesting that such a division is correlated with the presence of two testes around the site of bifurcation. We speculate that the loss of the left testis in Thaumastodermatinae (Ruppert 1978b) might be functionally correlated with the loss of this muscle bifurcation.

Phylogenetic analyses also may suggest other functional questions such as: why is the site of anterior ventrolateral muscle insertion correlated with the presence of anterior adhesive tubes, and do changes in the positions of both muscle insertion and adhesive tubes affect locomotory ability? Anterior adhesive tubes presumably function to stabilize the head of an animal during turbulent water flow (personal observations), and the close proximity of muscle insertion may increase this stability. Potentially, the closer the site of muscle insertion and adhesive tubes to the mouth rim, the more ventral surface area that is in contact with the substrate, corresponding to decreased lift from turbulent water flow.

If this scenario is plausible, then the selective factor for this change could be hydrodynamic in nature, and we may expect certain clades to have evolved under special hydrodynamic and sedimentary conditions (high wave energy vs. low wave energy; large grain size vs. small grain size, etc).

Trends in the muscular system of gastrotrichs are likely to become more evident with increased sampling of taxa not analyzed in this study (Planodasyidae, Lepidodasyidae). The need for further research on these families has been alluded to previously (Hochberg & Litvaitis 2000c, 2001c), and it is likely that the polyphyletic nature of both groups will be resolved with increased attention to their muscular system. Further, an analysis of the Dactylopodolidae is important to efforts to understand the origin and evolution of gastrotrichs and the function of their muscular system, an understanding currently impeded by a lack of knowledge about the most primitive clades. We suggest that attention be directed toward these genera, and those not mentioned in this study, with detailed observations of behavior, to achieve a greater understanding of the functional morphology of muscles in this phylum.

# CHAPTER 4

## FUNCTIONAL MORPHOLOGY OF THE MUSCLES IN *TETRANCHYRODERMA PAPII* (GASTROTRICHA, MACRODASYIDA)[1]

### Introduction

Locomotion in gastrotrichs is generally accomplished by ciliary gliding, whether the animals live in the interstitial environment or have an epiphytic or semi-pelagic existence. Movement patterns in different species of Gastrotricha were described early on by Zelinka (1889) and Remane (1933, 1935-1936), as were the cilia and muscles that contribute to these patterns (Remane 1935-1936). Since then, information on gastrotrich musculature has increased (Teuchert 1974, 1978; Ruppert 1982; Teuchert and Lappe 1980; Travis 1983) but knowledge of muscle function has received limited attention (Ruppert and Travis 1983; Ruppert 1991). Body-wall muscles are either oblique or cross-striated bands present in circular, longitudinal or, rarely, dorso-ventral orientations (reviewed by Ruppert 1991). In general, circular muscles lie internal to longitudinal bands along the pharynx, whereas the opposite arrangement occurs on the intestine. Other sets of muscles may include helicoidal bands around the entire digestive tract (Hochberg and

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[1] Hochberg, R. & Litvaitis, M.K. 2001. Functional morphology of the muscles of *Tetranchyroderma papii* (Gastrotricha, Macrodasyida). Zoomorphology 121:37-43.

Litvaitis 2001a). Various longitudinal muscles, often spanning the length of an animal, perform flexion in the dorso-ventral and lateral planes, and presumably also contribute to the characteristic whole-body flexion observed during mating (see Teuchert 1968; Ruppert 1978a). Escape responses are well known for a few genera, wherein body wall muscles and adhesive tubules are used successively in a rearward hyperextension, attachment, withdrawal motion to inchworm the animal backward along the substrate (Ruppert 1991); cilia presumably contribute only to forward movement.

Unlike their use in the Platyhelminthes (see Tyler and Hyra 1998; Hooge and Tyler 1999a), whole-body muscle patterns in gastrotrichs have yet to be used in taxonomy, and have received limited attention in phylogeny reconstruction (Ruppert 1982; Travis 1983). In some cases, the presence or absence of particular muscle groups have provided insight into the evolution of gastrotrichs, and ultimately their intraphyletic relationships (Ruppert 1982; Travis 1983). Knowledge of the function and the reason for the particular orientation of these muscles may enhance their utility in phylogeny. The overall aim of this paper was to identify the muscles of a well-known marine gastrotrich, *Tetranchyroderma papii* Gerlach, 1953 to understand how these muscle patterns contribute to the animal's behavior, and determine how these muscles may be used in systematic classifications.

## Materials and Methods

Specimens of *Tetranchyroderma papii* Gerlach, 1953 (Fig. 4.1) were collected from Hampton Harbor, New Hampshire (70° 49′ 13″ W, 42° 53′ 43″ N). Animals were present in low numbers around mid-tide level in medium to fine grain sand. Gastrotrichs

were extracted from the sediment using an anesthetization/decantation technique with 7.5% magnesium chloride (Pfannkuche & Thiel 1988). Specimens were kept in petri dishes of ambient seawater for 24 hrs and observed under a dissecting microscope for movement patterns.

For whole-mount muscle staining, animals were relaxed for 10 minutes in 7.5% magnesium chloride solution prior to fixation in 4% formaldehyde in 0.01M PBS (1 hr). Animals were rinsed in 0.01M PBS, permeabilized for 1 hr in 0.2% Triton X-100 in PBS, stained 40 min with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR) and rinsed in PBS before mounting with Gel/Mount (Tyler and Rieger 1999; Hochberg and Litvaitis 2000a). Specimens were viewed on a Zeiss epifluorescence microscope equipped with Spot Cooled Color digital camera (Diagnostic Instruments, Inc.). Measurements of gastrotrichs were performed with an ocular micrometer and the positions of particular organs are expressed in reference to percentage body units (total body length being 100 units (U)).

## Results

### General Musculature

Muscles associated with the body-wall, digestive tract, and reproductive organs stained with Alexa Fluor 488 phalloidin. Muscle fibers on the wall of the alimentary tract were found in circular, longitudinal, and helicoidal orientations. All three muscle types also supplied the oral hood (Fig. 4.2B). Longitudinal muscles were the only somatic component in the trunk region (Figs. 4.2-4.5). Muscles associated with the male caudal

organ were present in circular and longitudinal orientations. Unless otherwise stated, muscle locations are given with reference to adult animals approximately 325 µm long.

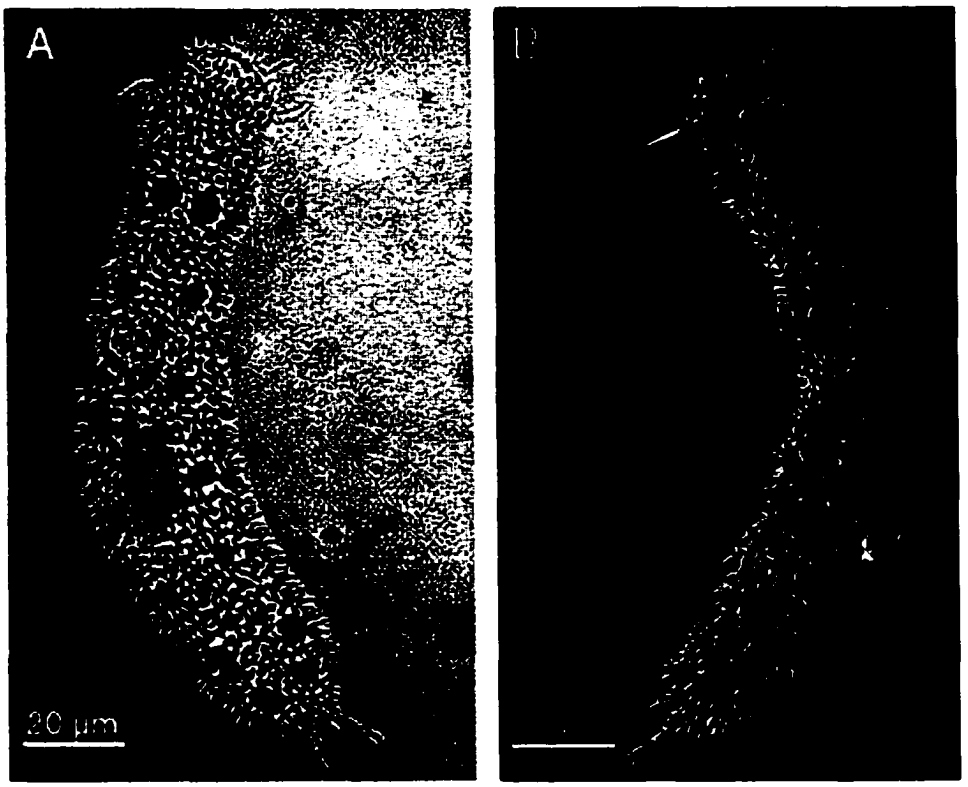


Figure 4.1. *Tetranchyroderma papii* in dorsal view. A, Light micrograph. B, SEM micrograph.

Splanchnic Circular Muscles

The circular muscles were present only in splanchnic positions. Somatic circular muscles, present as lateral branches from the splanchnic circular muscles along the intestine, were absent from this species. Individual muscle rings on the pharynx and intestine were < 1µm-2 µm in width and did not appear to branch. Apically, several

muscle rings contributed to the structure of the oral hood (Fig. 4.2B). In an adult gastrotrich, approximately 20 individual circular muscle fibers supplied the oral hood. Circular muscle rings in the dorsal hood arched ventrally and caudally to encircle the mouth. The first set of muscle rings against the fringe of the oral hood and mouth are compact, forming a distinct sphincter. Circular muscles become more evenly spaced posteriorly along the pharynx. Approximately 9 individual circular bands could be counted in a 10 µm longitudinal view of the pharynx. Circular rings were present along the entire pharynx, with a slight gap between rings at the pharyngeal pores (Fig. 4.3). The pharyngeal pores appeared as lateral tube-like extensions connecting the pharynx to the body-wall, but distinct muscles around the extensions were not evident. The circular muscles became more dispersed along the intestine, with approximately 5 rings per 10 µm (Figs. 4.3, 4.4).

A single extraordinary semi-circular muscle band (SCM) was found ventrally at U77 (Fig. 4.5A, 4.6A). This band (4 µm wide) extended between two large ventrolateral longitudinal muscle bands. The band was located ventral to the intestine and reproductive organs and there was no dorsal counterpart to this muscle.

Splanchnic Longitudinal Muscles

Longitudinal muscles were the thickest muscles in the body (up to 5 µm wide) but less numerous than the circular fibers. Several dorsal, lateral and ventral muscle bands extended the length of the animal. Distinguishing between somatic and splanchnic longitudinal muscles, however, was difficult in this species. On the pharynx, dorsal

longitudinal muscles were exterior to circular muscles, whereas the condition was reversed along the intestine.

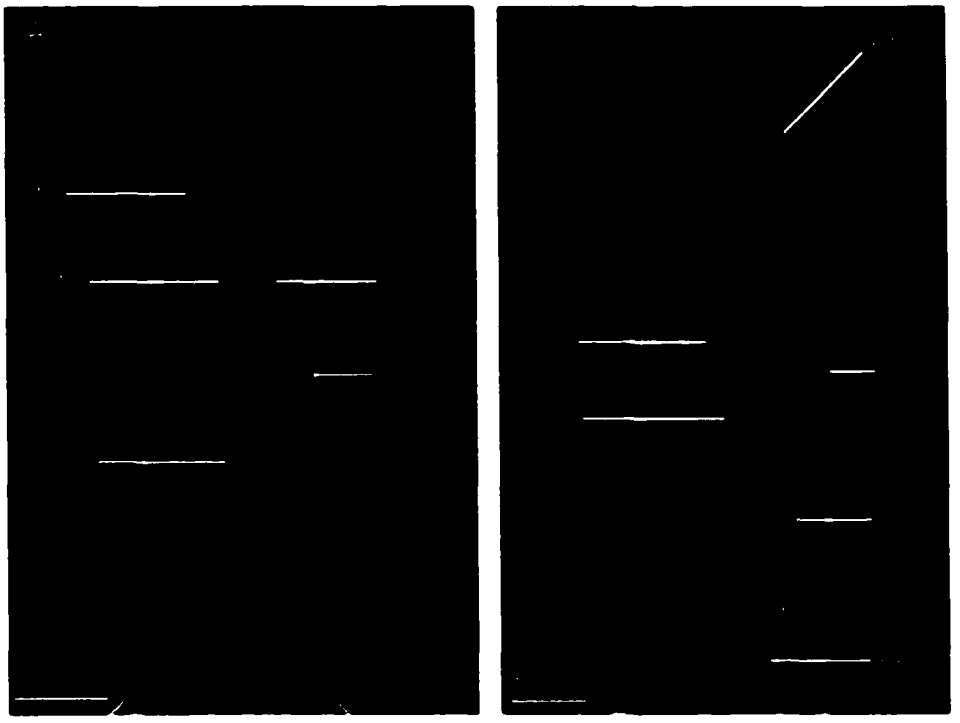

Figure 4.2. Phalloidin-stained specimens of *T.papii* revealing muscles. A, Ventral view of anterior end. B, Dorsal view of anterior end. *CM* circular muscle of oral hood, *DMM* dorsal median muscle, *EG* epidermal gland, *HM* helicoidal muscle, *LB* ventral longitudinal muscle, *MB* ventral median muscle, *PH* pharynx, *SM* sphincter muscles, *VLM* ventrolateral muscle

The only longitudinal muscles that never appeared to be surrounded by circular bands were the ventrolateral longitudinal muscles. Two pairs of dorsal bands extended from the oral hood to the caudal end. These bands were difficult to follow along the length of the body owing to their thin diameter (1-2 μm). At the anterior end, the most medial muscle pair bifurcated three times, once at approximately U12, and again at U7 and at the hood

rim (Figs. 4.2A, 4.6B).The more lateral muscle pair branched in a similar way to the medial muscle pair, but specific branching locations were not visible. In the trunk region, the muscle pairs remained separate. At the caudal end, the medial and lateral muscle bands on each side of the midline appeared to coalesce into two bands that inserted at the body midline.

Two pairs of longitudinal muscles were present along the ventral aspect of the pharynx (Figs. 4.2B, 4.6A). Anteriorly, both muscle bands inserted on the ventral mouth rim. Approximately 5-10 μm posterior to the mouth rim, the muscle pairs coalesced into a single band, only to separate again. The most medial separation (MB) was located close to the midline of the body. The second pair (LB) was in a more lateral position.

Five pairs of muscle bands were present along the intestine (Figs. 4.3, 4.4, 4.6). Two muscle pairs (MB & LB) were continuations of pharyngeal muscles. The lateral muscle band (LB) extended toward the anus, producing a single lateral branch once (TLB) at U33 that ran parallel to LB. The medial muscle band (MB) from the pharynx tapered and terminated on the intestine at U39. A thin muscle band (MMB) originated medial to MB at U36 and terminated at the anus. A fifth pair of muscle bands (MMM) originated at U50, medial to MMB, and continued toward the anus. Muscles appeared to anastomose caudally and insert as two individual bands close to the anus.

Splanchnic Helicoidal Muscles

The thinnest muscles in the body were the helicoidal muscles (< 1 μm), lying entirely along the alimentary tract and providing only a small contribution to the oral hood (Figs. 4.2, 4.3, 4.5B). Helicoidal muscles appeared to consist of at least two

individual muscle bands crossing each other in a double-helix fashion. The helicoidal muscles appeared to enwrap the circular muscle bands, but it was not possible to determine if they also enwrapped the longitudinal muscle bands (dorsal and ventral) of the pharynx. Helicoidal muscles did not appear to encircle the ventrolateral longitudinal muscles. The helicoidal muscles overlapped one another to some extent and did not form perfect crosses in either dorsal or ventral views. There were approximately 4 helicoidal crosses surrounding the pharynx and up to 10 crosses surrounding the intestine in an adult specimen (330 μm). Juveniles had fewer helicoidal crosses. Dorsally, a single helical cross supplied the lateral margins of the dorsal hood.

Somatic Longitudinal Muscles

The thickest of the longitudinal muscles (4–6 μm) were the ventrolateral bands (VLB), which ran parallel to the gut tube but did not lie entirely against it (e.g., compare splanchnic muscles). These muscles extended from the lateral mouth margin to the caudal peduncle. At the mouth rim, these muscles branched several times, some branches inserting at the mouth rim and others appearing to supply lateral portions of the oral hood. Those that supplied the hood were extremely thin (< 1 μm). Posteriorly, the ventrolateral muscle bands remained separate and without noticeable bifurcation until approximately U77, where a thinner muscle band crossed over diagonally to the opposite side of the body, supplying the contralateral muscle band (VLM) at U90 (Figs. 4.4, 4.5A). Where the crossover muscles met they formed a 45-50° angle with respect to the longitudinal body axis. At U90, the ventrolateral muscle bands split into 2 major

divisions. The most lateral division supplied the caudal peduncle, inserting at the proximal end of the adhesive tubes (Figs. 4.4, 4.5A). The inner division had three branches: lateral,

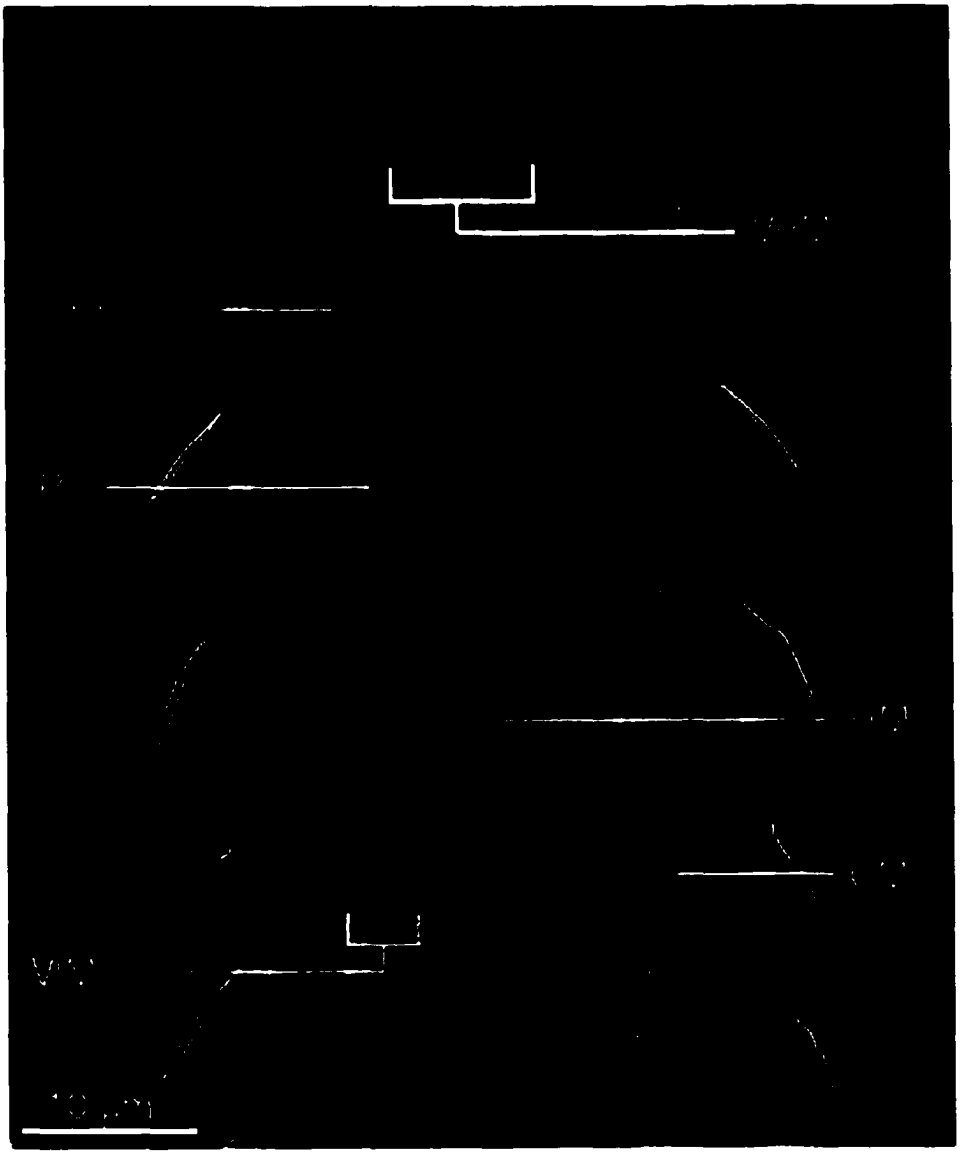

Figure 4.3. Phalloidin-stained specimens of *T.papii* revealing muscles. A, Ventral view of anterior end. B, Dorsal view of anterior end. *CM* circular muscle of oral hood, *DMM* dorsal median muscle, *EG* epidermal gland, *HM* helicoidal muscle, *LB* ventral longitudinal muscle, *MB* ventral median muscle, *PH* pharynx, *SM* sphincter muscles, *VLM* ventrolateral muscle.

median, and interior. The lateral branch (LLB) inserted on the medial aspect of each half of the caudal peduncle. The median branch (LMB) inserted at the midline of the trunk. The most interior branch (CMS) crossed over the body midline and supplied the opposite branch of the caudal peduncle, inserting on its medial aspect. The angle between these crossover muscles was approximately 45° to the longitudinal body axis.

Locomotion and Behavior

Four general forms of movement characterized *T.papii*: ciliary gliding, head waving, forward creeping, and backward creeping. The animal glided along the surface of sand grains using its ventral locomotory cilia. Normal forward movement progressed at a rate of 200–330 μm/second. Directional changes were made by lateral bending of the body. Occasionally, the animal would stop forward locomotion and engage in a form of head waving behavior. In this instance, the caudum was pressed against the substratum and the head end was raised and rotated dorsolaterally, torting the anterior end (Fig. 4.6). This behavior generally lasted less then 5 seconds and was followed by normal forward progression. Similar dorso-ventral flexion of the tail occurred at random.

Movements in response to harmful stimuli (prodding with a micropipette) took the form of creeping behavior. In backward creeping, ciliary activity stopped, ventrolateral adhesive tubes were attached to the substrate, the caudal end hyperextended backwards attaching the caudal adhesive tubes, and the body was withdrawn. The posterior end was then re-extended and the cycle repeated. This backward withdrawal rarely went beyond two cycles before a subsequent direction change and normal ciliary gliding. Another form of creeping was often observed, though rarely in response to the

same stimuli. This behavior involved forward creeping and resulted in inchworm-like movement. In this behavior, the anterior adhesive tubes were pressed against the substrate, caudal and lateral adhesive tubes released from the ground, and the trunk was drawn forward to the head (body may contract to nearly 3/4 its original length). Re-extension of the anterior trunk occurred immediately. Forward creeping may repeat up to three times in succession.

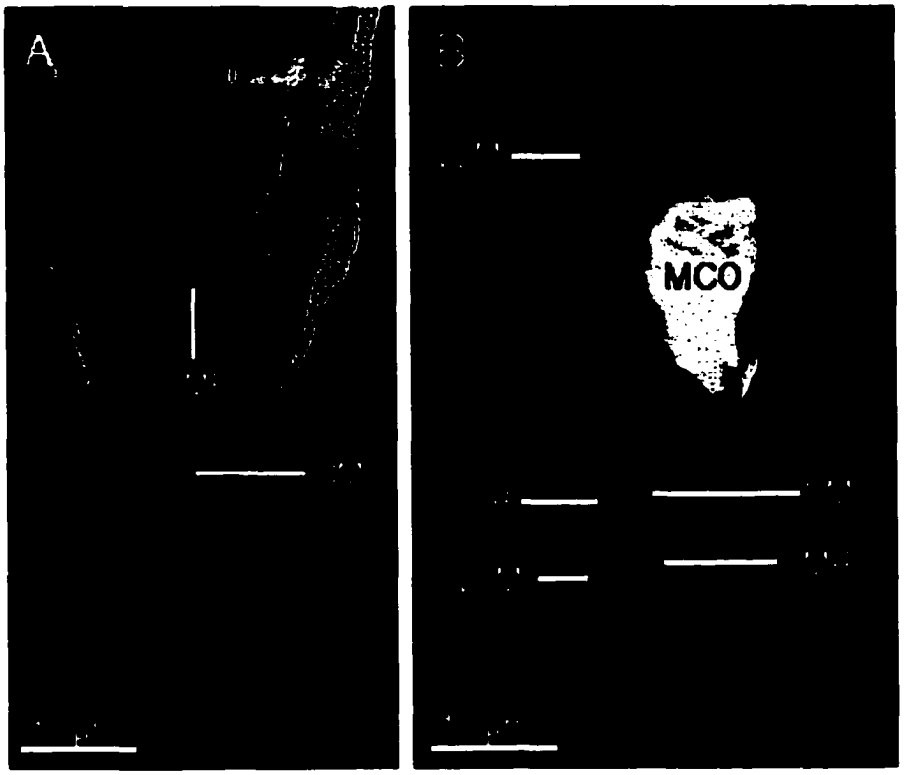


Figure 4.4. Posterior end of *T.papii* in two focal planes. A, Ventral view in shallow focal plane. B, Ventral view in deep focal plane. *CMF* first crossover muscle, *CMS* second crossover muscle, *LLB* lateral branch of ventrolateral muscle band, *LMB* median branch of ventrolateral muscle band, *MCO* male caudal organ, *SCM* semi-circular muscle band, *VLM* ventrolateral muscle band.

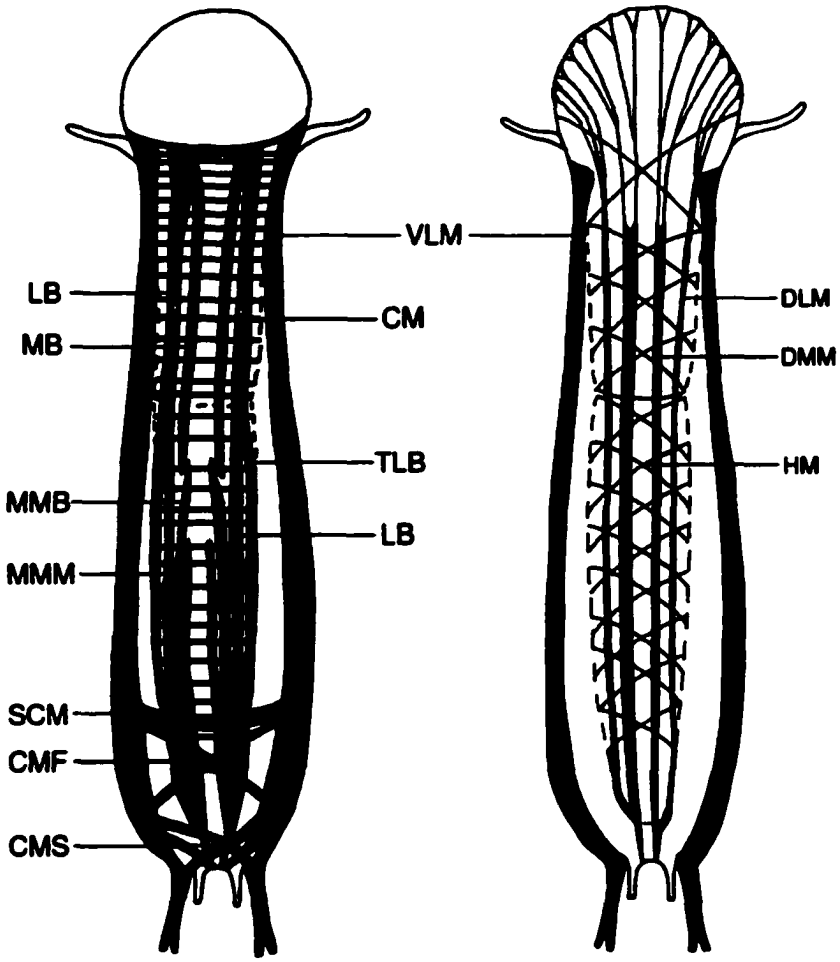


Figure 4.5. Schematic drawing of *T.papii* revealing muscles visualized with phalloidin staining. A Ventral view, helicoidal muscles omitted. B Dorsal view, circular muscles omitted. *CMF* first crossover muscle, *CMS* second crossover muscle, *DLM* dorsal lateral muscle, *DMM* dorsal median muscle, *HM* helicoidal muscle, LB lateral muscle band of alimentary canal, *LLB* lateral branch of ventrolateral muscle band, *LMB* median branch of ventrolateral muscle band, *MB* medial muscle band of alimentary canal, *MCO* male caudal organ, *MMB* thin medial muscle to *MB*, *MMM* most medial muscle band, *SCM* semi-circular muscle band, *VLM* ventrolateral muscle band.

## Discussion

### Muscle Patterns

This study represents the first detailed functional examination of the muscles in a marine gastrotrich using fluorescently labeled phalloidin. Similar research on muscle patterns has been performed on rotifers (Hochberg & Litvaitis 2000a) and a variety of Plathelminthes (Rieger et al. 1994; Tyler and Hyra 1998; Hooge and Tyler 1999a, b; Tyler and Rieger 1999). However, unlike similar-sized flatworms, gastrotrichs differ in their muscle organization by possessing distinct band-like muscles oriented along the alimentary tract, as opposed to the grid-like network of fine muscle fibers composing the body wall of platyhelminths. Apart from ultrastructural observations on striation pattern and cell shape (Teuchert 1974, 1977; Teuchert and Lappe 1980, Ruppert 1991), no formal terminology has been used in the description and classification of musculature of macrodasyidan gastrotrichs (see Ruppert 1975 for musculature of Chaetonotida).

The distribution of muscles in *T.papii* is in general agreement with those visualized in other species using TEM (Teuchert and Lappe 1980; Travis 1983). Moreover, the concentration of longitudinal muscles on the ventral side of *T.papii* is similar to that seen in *Thaumastoderma heideri* Remane, 1926 and *Pseudostomella roscovita* Swedmark, 1956 (personal observations) and in members of Lepidodasyidae and Turbanellidae (Travis 1983). In *Dactylopodola baltica* (Remane, 1926) though, a complete ring of longitudinal muscles is found (Travis 1983). The complete dorsal and ventral musculature is thought to represent the plesiomorphic condition for the Macrodasyida, and thus, its absence in *T.papii* indicates a derived status. Somatic circular muscles, present as lateral

branches of splanchnic circular rings in the trunk region (Teuchert & Lappe 1980), were absent in this species, and is another indication of the derived condition of *T.papii*. It is noteworthy that somatic circular muscles have not been found to exist without their splanchnic counterpart in Macrodasyida, though the reverse condition is well known (Ruppert 1978b; Travis 1983).

Noteworthy among the muscles present of *T.papii* are the crossover muscles, helicoidal muscles, and a single, semi-circular band. The presence of two crossover muscles is unique to *T.papii*, though a single pair of bands is present in other members of the genus as well as *Pseudostomella roscovita* and *Thaumastoderma heideri* (personal observations). Similar crossover muscles are also found in *Dactylopodola baltica* and *Turbanella cornuta* Remane, 1926 (see Travis 1983) though their structure and location are slightly different from *T.papii* (personal observations). The presence of this unique muscle orientation in the same general location among different genera may be indicative of close phylogenetic relations or similar selective pressures in the interstitial environment.

Helicoidal muscles have been found in several species of Gastrotricha, though the number of bands and their precise location may vary (Hochberg and Litvaitis 2001a). The function of these unique muscles is undetermined (see below), but their wide-spread systematic distribution indicates they are likely part of the ground pattern of the Gastrotricha.

Last of the extraordinary muscles of *T.papii* is the presence of a single, semi-circular muscle band ventral to the intestine and reproductive organs. This is a unique find

for the species, and to our knowledge has not been identified in any other gastrotrich. The thickness of the band suggests it is not likely to be an evolutionary remnant of the lost circular musculature, considering circular fibers in gastrotrichs are fairly thin and, at least dorsally, never incomplete (Hochberg and Litvaitis 2000a). Interestingly, this band is also absent from other members of the genus and the family (personal observations) suggesting a unique local adaptation.

Muscle Organization and Movement

The functions of the observed muscles in *T.papii* are inferred based on their position and the known movement patterns of the animal. *T.papii* is an extremely mobile animal that shows high flexibility and very characteristic movement patterns. Normal forward locomotion is accomplished by ciliary gliding, and turning movements are created through alternate contractions of the ventrolateral muscle bands. The complexity of the cuticle does not appear to impede bending in this species. On the contrary, species of *Tetranchyroderma* appear nearly as flexible as the more elongate forms with smooth cuticles (*Dolichodasys elongatus* Gagne, 1980; personal observations). As noted by Travis (1983), the complex cuticle and Y-organ of species of *Tetranchyroderma* cf. *swedmarki* Lévi, 1959 may serve as the primary antagonists to the ventrolateral longitudinal muscles of the trunk region, considering the lack of circular body-wall muscles surrounding them. The presence of a Y-organ in *T.papii* is unknown. The high flexibility of *T.papii* is best observed when it characteristically glides along sand grains, often pausing to dorso-flex the anterior end, followed by a helical sweep of the head (Fig. 4.6). On the basis of muscle position, this behavior is likely to be accomplished by the

thin dorsal longitudinal muscles, which are maintained in a contracted state while a single (left or right) ventrolateral muscle band contracts. An ultrastructural analysis of this species in contracted vs. relaxed states should help clarify the roles of various muscles and skeletal elements (cuticle, Y-organ) in general body flexion and movement.

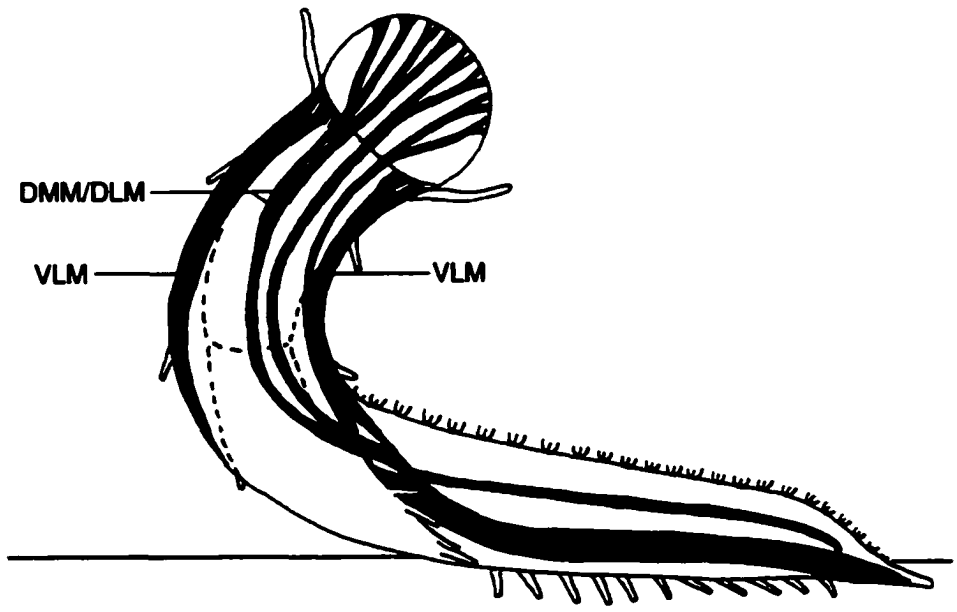


Figure 4.6. Schematic drawing of *T.papii* during torsion of the anterior end and the muscles that contribute to this behavior. *DMM* dorsal median muscles, *DLM* dorsal lateral muscles, *VLM* right and left ventrolateral muscle bands.

While turning and bending appear to be the fundamental function of the longitudinal muscle tracts, other extraordinary muscle groups may serve different functions. In the posterior region of the body, there are two sites of crossover muscles where longitudinal fibers from the main ventrolateral bands cross over to the opposite caudal lobe. The first crossover pair occurs close to the reproductive organs, and the second pair occurs post-anal. The function of these crossover muscles is unknown, but we hypothesize that they may serve to stabilize the posterior end against the substrate, bringing the posterior, sublateral adhesive tubules in contact with the ground.

The presence of other muscle bands closely associated with the digestive tract is indicative of their function. Circular muscles only exist along the digestive tract, and as suggested by Ruppert (1982, 1991), their function appears to be as antagonists to dilations of the pharynx and intestine during feeding. *T.papii* regularly consumes relatively large pennate diatoms and so requires a propulsive force to move food items through the digestive tract. We hypothesize that, as in other species with helicoidal muscles (Hochberg & Litvaitis 2001a), the function of these muscles is to assist the circular muscles in counteracting the pharyngeal and intestinal dilations. A second potential function of helicoidal muscles may be to stiffen the gut, thereby adding rigidity to the body.

The function of the single, semi-circular muscle band in the posterior body wall of *T.papii* is difficult to define. It is not likely an antagonist to the longitudinal muscles due to its incomplete structure. Instead, the position of the band beneath the reproductive organs might be indicative of a role during mating. An ultrastructural examination of this

muscle and its presumed connection with reproductive organs and other closely positioned muscles appears warranted.

The muscular system of *T.papii* shares several similarities with other members of the Macrodasyida. A complete map of the muscle systems within the Macrodasyida is likely to contribute important information on the adaptive value of particular muscle groups to animals in different environments (interstitial, epibenthic, semi-pelagic), especially when combined with ultrastructural data of the skeletal system. In addition, muscle patterns may contribute important information toward a greater understanding of gastrotrich evolution and phylogeny, as they have for some turbellarians (see Rieger et al. 1994; Tyler and Hyra 1998; Hooge and Tyler 1999b; Hooge 2001).

# CHAPTER 5

## A MUSCULAR DOUBLE-HELIX IN GASTROTRICHA[1]

### Introduction

The morphology of the muscular system of gastrotrichs has been examined in some detail at the light microscopic (Remane 1929, 1935-1936) and transmission electron microscopic levels (Teuchert 1974, 1977; Teuchert & Lappe 1980; Ruppert 1982, 1991; Travis 1983). By means of serial thin sections, major components of the musculature of several species have been reconstructed, providing a detailed view of their ultrastructure and arrangement (Ruppert 1991). The gastrotrich muscular system comprises a series of hoop-like circular muscles around the digestive tract, surrounded by individual bands of longitudinal musculature, and finally somatic circular muscles. One or more of these muscle arrangements may be missing in particular species (Remane 1935-36; Travis 1983). Resolving this internal musculature has relied primarily on sectioned material, and consequently, knowledge of the orientation of specific muscle groups is dependent upon serial reconstruction of TEM micrographs. Other techniques to view the musculature involve the use of fluorescent phallotoxins to visualize F-actin-containing tissues (Wulf et

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1. Hochberg, R. & Litvaitis, M.K. 2001. A muscular double-helix in Gastrotricha. Zoologischer Anzeiger 240: 59-66.

al. 1979). Whole-mount methods using fluorescent-labeled phalloidin have been employed to view whole muscle systems in various micrometazoans including members of the Platyhelminthes (Rieger et al. 1991, 1994; Mair et al. 1998; Tyler & Hyra 1998; Hooge & Tyler 1999a, 2000) and Nematoda (Francis & Waterston 1985; Priess & Hirsh 1986). These studies revealed the complexities of muscle arrangements, especially in turbellarian flatworms, and have provided important information for systematic and phylogenetic purposes (Tyler & Hyra 1998; Hooge & Tyler 1999b; Hooge 2001).

We have used similar techniques to visualize the muscle patterns in a variety of marine and freshwater Gastrotricha with the goal of achieving a better understanding of their functional morphology. During these investigations, we came across a muscle arrangement currently unknown for gastrotrichs. Helicoidal muscles, in left- and right-hand spirals around parts of the digestive tract, were found in several species of benthic gastrotrichs. We describe the position and orientation of the helicoidal muscles and postulate their function.

## Materials and Methods

Seabrook Beach, New Hampshire, USA was the site of collection for the following macrodasyidan gastrotrichs: *Acanthodasys aculeatus* Remane, 1927, *Macrodasys caudatus* Remane, 1923, *Tetranchyroderma papii* Gerlach, 1953, *Thaumastoderma heideri* Remane, 1926, and *Turbanella cornuta* Remane, 1925. The marine chaetonotidans, *Chaetonotus aculifer* (Gerlach, 1953), *Draculiciteria tessalata* (Renaud-Mornant, 1968), and *Xenotrichula intermedia* Remane, 1934, were collected from Hampton Beach, New Hampshire. All marine gastrotrichs were present in low numbers around mid - tide level in

medium-to fine - grain sand (5 cm depth). Gastrotrichs were extracted from the sediment using an anesthetization-decantation technique with 7.5% magnesium chloride (Pfannkuche & Thiel 1988). *Lepidodermella squamata* (Dujardin, 1841) was obtained from isolation culture (Connecticut Valley Biological Supply, Northampton, Massachusetts).

For whole-mount muscle staining, marine gastrotrichs were relaxed for 10 min in 7.5% $MgCl_2$, and freshwater gastrotrichs were relaxed in 1% $MgCl_2$. Following 1 hr fixation in 4% formaldehyde in 0.01M phosphate buffered saline (PBS), animals were rinsed in 0.01M PBS, permeabilized for 1 hr in 0.2% Triton X-100 in PBS, stained for 40 min with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR), and rinsed in PBS before mounting with Gel/Mount (Biomeda Corp.). Specimens were viewed on a Zeiss Axiophot epifluorescence microscope equipped with a Diagnostic Instruments SPOT Cooled Color digital camera.

## Results

General morphological descriptions of all examined genera can be found in Remane (1929, 1935-1936) and Ruppert (1979, 1988). Patterns of musculature of species in this study, including somatic and sphlancnic muscles, were all made visible with the phalloidin stain. The layering of the muscles could often be discerned with optical sectioning.

### Order Macrodasyida

The musculature of all macrodasyidans included at least three layers from digestive tract to body-wall: sphlancnic circular muscles, longitudinal muscles, and helicoidal muscles. The number of muscle layers and the their position often differed

between regions of the body (pharynx vs intestine) and among species. The pharynx of all species was lined by sphlancnic circular musculature that consisted of numerous, individual hoop-like bands. Longitudinal muscles were present outside the circular bands and lay directly against them in dorsal, ventral and ventrolateral positions. Dorsal and ventral muscles always inserted at the anterior end of the pharynx, whereas the larger ventrolateral bands inserted either at the mouth rim (*Acanthodasys aculeatus*, *Macrodasys caudatus*, *Tetranchyroderma papii*, *Thaumastoderma heideri*) or ventral adhesive tubes (*Turbanella cornuta*). Sphlancnic circular muscles lined the intestine, but the position of longitudinal bands (inside or outside) relative to the circular muscles was often difficult to distinguish. Somatic circular muscles enclosing the ventrolateral muscles were present only in *M. caudatus* and *T. cornuta*. Posteriorly, longitudinal bands lining the digestive tract inserted on the posterior portion of the intestine or at the body midline. Ventrolateral muscles inserted posteriorly behind the anus and close to the body midline (*M. caudatus*) or in the caudal lobes (*A. aculeatus*, *T. papii*, *T. heideri*, *T. cornuta*). Helicoidal muscles were always the thinnest-diameter muscles in the body ($\leq$ 1µm) and appeared to surround sphlancnic circular and longitudinal muscle bands in all species examined (Figs. 5.1-5.3). Most helices overlapped one another, and muscle fiber angle was 40-60° with respect to the longitudinal axis of the body.

In *Acanthodasys aculeatus*, *Tetranchyroderma papii*, and *Thaumastoderma heideri*, helicoidal muscles were present along most of the digestive tract, and in *T. papii* and *T. heideri*, made a small contribution to the dorsal oral hood (Figs. 5.1, 5.2). In a 300 µm-long specimen of *T. papii*, helices crossed each other approximately 4 times along the

length of the pharynx and 10 times along the length of the intestine. In *M. caudatus* and *T. cornuta*, helicoidal muscles were found mainly on the pharynx (Figs. 5.2, 5.3), with up to 8 crosses spanning the length of a 250 μm pharynx in *M. caudatus* (individual body length = 550 μm). Also in each species, a single helix was seen below the pharyngeal-intestinal junction. Precise points of helicoidal muscle insertion were undetermined.

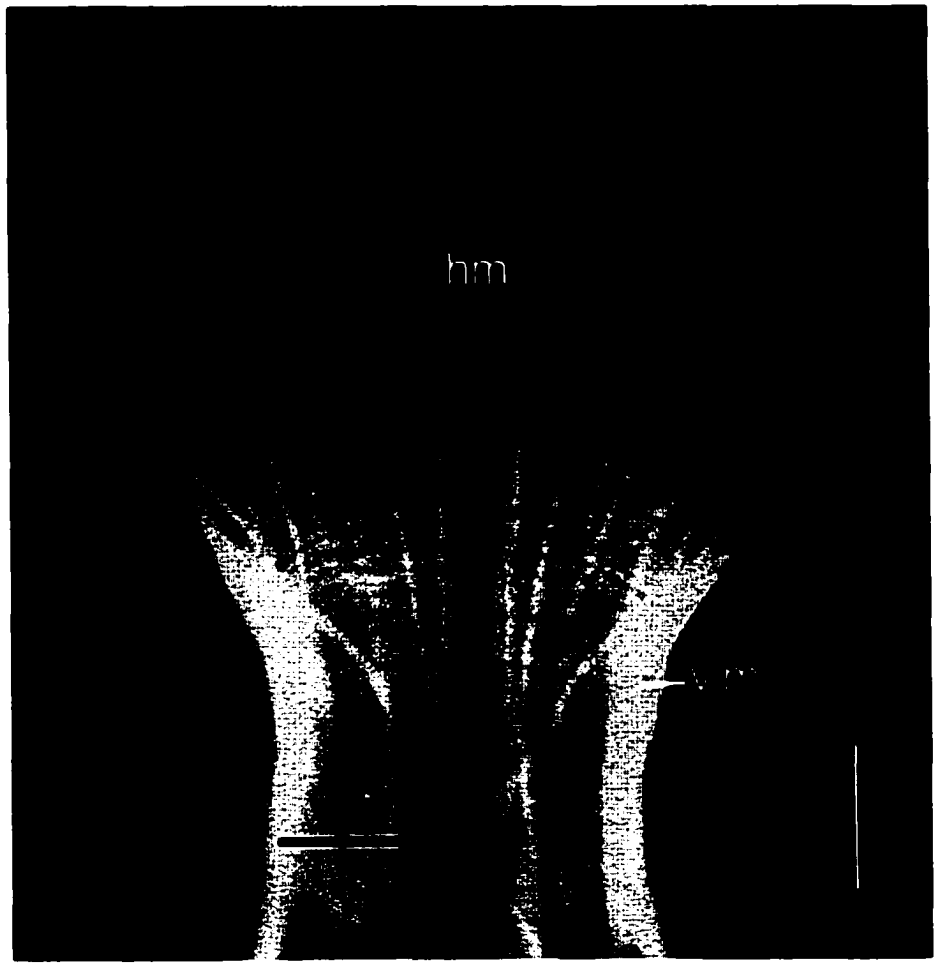


Figure 5.1. Dorsal view of oral hood of *T. papii*. arrow = helicoidal muscle, hm = hood muscles. Scale bar = 10 μm.

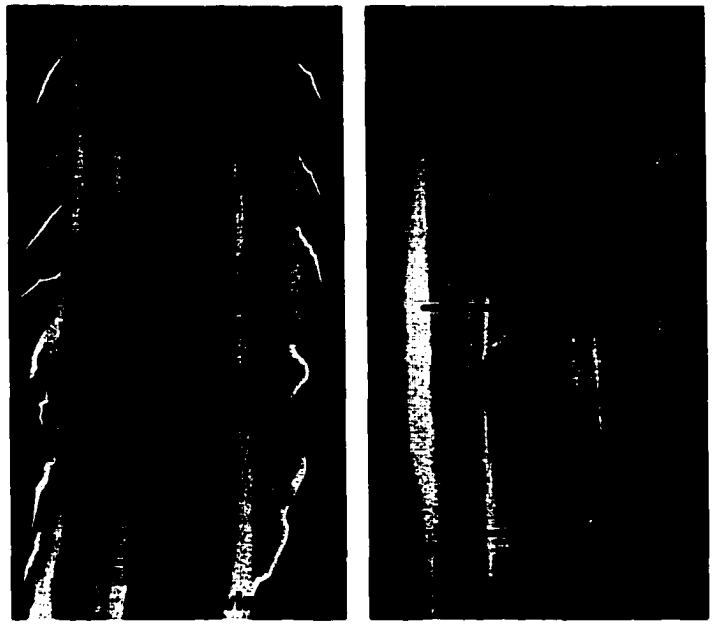

Figure 5.2. Ventral views of macrodasyidan gastrotrichs. Left: mid-trunk of *T. papii*. arrow = helicoidal muscle, icm = intestinal circular muscles, ilm = intestinal longitudinal muscles, vlm = ventrolateral longitudinal muscle. Right: pharynx of *M. caudatus*. arrow = helicoidal muscle, cm = circular muscle, lm = longitudinal muscle, vlm = ventrolateral longitudinal muscle. Scale bar = 15 μm.

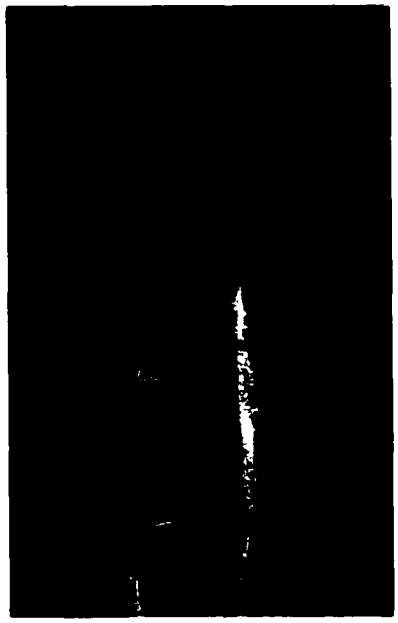

Figure 5.3. Ventral view of pharynx of *Acanthodasys aculeatus* showing helices (arrows) and ventrolateral muscle (vlm). Scale bar = 20 μm.

Order Chaetonotida (Suborder Paucitubulatina)

The musculature of *Chaetonotus aculifer, Draculiciteria tessalata Lepidodermella squamata,* and *Xenotrichula intermedia* consisted of circular, longitudinal and helicoidal muscle bands. In *C. aculifer* and *L. squamata*, circular muscles were only present on the pharynx. In both species, longitudinal muscles were found in ventral, ventrolateral, and dorsal positions. Dorsal and ventral muscles inserted anteriorly in the mouth region and posteriorly at the body mid-line or on the ventrolateral muscles. The large ventrolateral bands inserted anteriorly at the mouth and posteriorly in the caudal furca. In *X. intermedia*, somatic circular muscles and sphlancnic circular bands were observed, though the latter muscles were difficult to resolve (Fig. 5.4). Circular musculature of *D. tessalata* was difficult to observe in the single specimen examined, but appeared to be found only in a somatic position. Dorsoventral muscles were noted for *D. tessalata.*

In the Xenotrichulidae (*D. tessalata, X. intermedia*), helicoidal muscles were present on the pharynx and intestine (Figs. 5.4, 5.5). Helicoidal bands on the pharynx were difficult to visualize in either species, so no accurate count of their crossing could be made. It was also undetermined whether these helicoidal bands enclosed the ventral longitudinal muscles in either species. In *D. tessalata*, helicoidal crosses were observed along the complete length of the intestine, whereas in *X. intermedia*, helicoidal crosses were observed only along the anterior portion of the intestine (Fig. 5.4). Fiber orientation of the helicoidal bands was approximately 55°. In *C. aculifer* and *L. squamata*, helicoidal muscles with fiber angles of 50-60° were present only along the intestine, and appeared to enclose the thin, ventral, longitudinal muscles (Fig. 5.5). Helices overlapped each other 5

times on the intestine of an adult *L. squamata* (160 µm). The center of each helix was spaced approximately 8-10 µm from the next. Helicoidal muscles were not present around the terminal portion of the intestine.

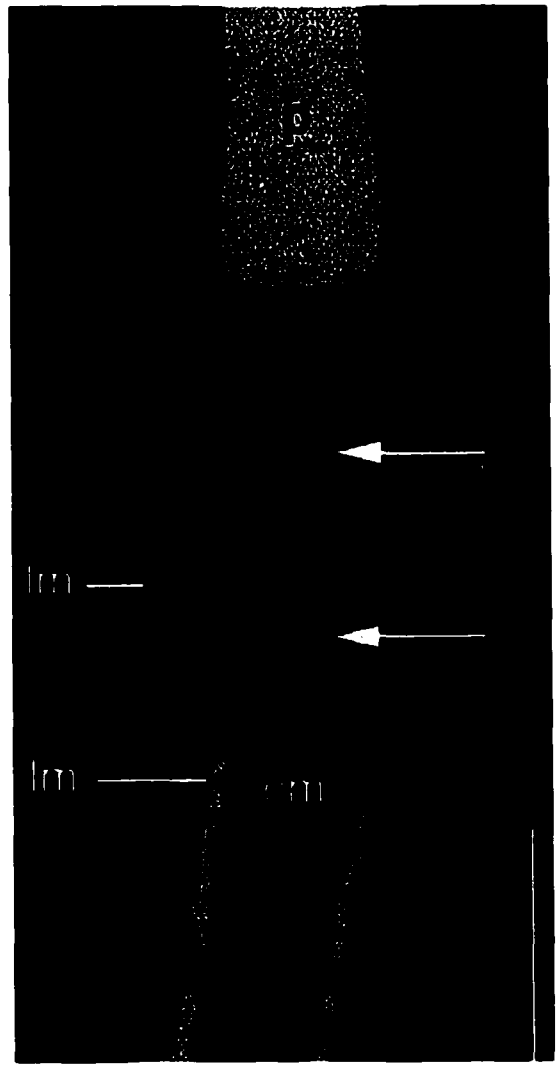


Figure. 5.4. Ventral view of helicoidal muscles below pharynx-intestine junction in *X. intermedia*. arrow = helicoidal muscle, cm = circular muscle, p = pharynx, lm = longitudinal muscle. Scale bar = 30 µm.

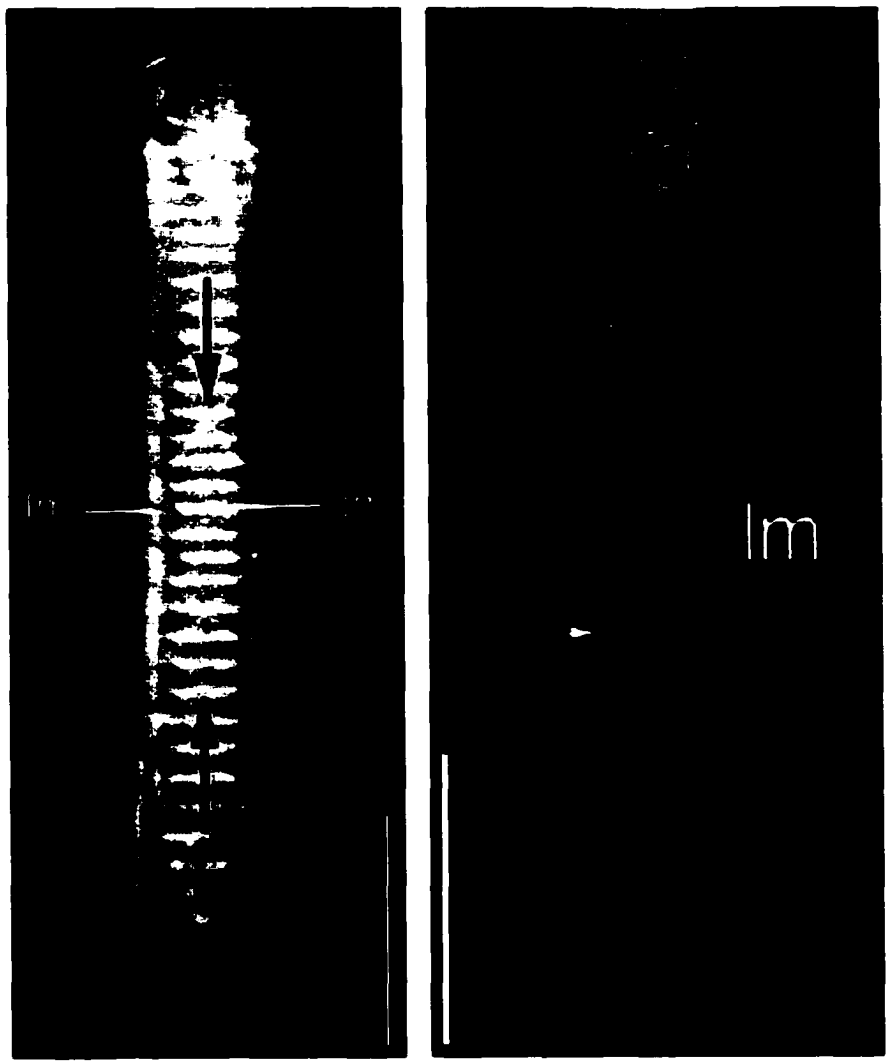


Figure 5.5. Ventral view of pharynx of two chaetonotidans. Left: pharynx of *D. tessalata*. arrow = helicoidal muscle, cm = circular muscle, lm = longitudinal muscle.Scale bar = 10 μm. Right: trunk of *L. squamata*. lm = longitudinal muscle on intestine, p = pharynx, arrow = site where helicoidal muscle enwraps longitudinal bands. Scale bar = 10 μm.

## Discussion

The muscular system of gastrotrichs is generally characterized as a stratified system of band-like muscles present in sphlancnic and somatic positions (Ruppert 1991). The sphlancnic musculature consists of numerous circular bands and closely aligned longitudinal muscles that line the digestive tract, whereas somatic components generally include large, ventrolateral, muscles enclosed by a separate ring of circular muscles. Dorso-ventral muscles compose a third muscle orientation found in some gastrotrichs (Ruppert 1979). Variations in these arrangements occur among species, and may have some bearing on phylogeny (Travis 1983). To these we can add a fourth muscle arrangement consisting of left- and right-handed (crossed) helicoidal muscles. We have used the term, helicoidal, to describe the orientation of these muscles with respect to the longitudinal body axis, instead of the term 'helical,' so as to avoid confusion with the description of fiber orientation within individual muscle cells (helical muscles, Lanzavecchia 1977).

The internal position of helicoidal muscles varies among the gastrotrich species examined. In the macrodasyidans, *Acanthodasys aculeatus*, *Thaumastoderma heideri* and *Tetranchyroderma papii*, the helicoidal muscles appear to spiral around both sphlancnic circular muscles and longitudinal bands on the pharynx and intestine. However, not all longitudinal bands were bound by these helicoidal muscles, namely, the large, ventrolateral bands used in general body flexion. In other macrodasyidans, *Macrodasys caudatus* and *Turbanella cornuta*, the helicoidal muscles were present mainly on the pharynx. Within the Chaetonotida, helicoidal muscles were also observed on the pharynx and intestine. In

members of the Xenotrichulidae, *Draculiciteria tessalata* and *Xenotrichula intermedia*, helicoidal muscles lined the entire digestive tract, though in *X. intermedia*, the muscles had a slightly more restricted distribution on the intestine (Fig. 5.4). In members of the Chaetonotidae, *Chaetonotus aculifer* and *Lepidodermella squamata*, where intestinal circular muscles are absent, helicoidal muscles only encircled the intestine and its associated ventral longitudinal musculature. Aside from the position of helicoidal muscles relative to other muscle groups, the number of helices appears to vary among taxa.

This is the first known account of helicoidal muscles in gastrotrichs. The discovery of this muscle orientation in gastrotrichs is surprising considering many of these species have also been examined via thin sections with the light microscope (Ruppert 1979) and electron microscope (Teuchert 1974, 1977; Teuchert & Lappe 1980; Ruppert 1991). However, the extremely thin nature of these muscles (< 1 μm) combined with their unusual orientation along the digestive tract, probably causes confusion with individual circular muscle bands in sectioned material.

The systematic distribution of helicoidal muscles in the Gastrotricha appears to be widespread, considering their occurrence among representatives of five families in two orders (Order Chaetonotida: Chaetonotidae, Xenotrichulidae; Order Macrodasyida: Macrodasyidae, Thaumastodermatidae, Turbanellidae). We found helicoidal muscles in all species examined, regardless of general body shape (elongate, ten-pin shape), cuticle morphology (smooth, flat scales, pentancreous hooks), or habitat type (marine, freshwater). It appears that this special muscle orientation may be a synapomorphy for

the phylum. Additional investigations on more primitive taxa (Dactylopodolidae, Neodasyidae) should further elucidate the ground pattern of these muscles.

The function of helicoidal muscles in gastrotrichs is unknown, but we hypothesize that their main role is in feeding mechanics and possibly conservation of intestinal shape during bending. The presence of this unique muscle arrangement on the digestive tract of all species suggests a similar functional role in species from both orders. Helicoidal muscles may function to pressurize the pharynx and intestine during feeding by antagonizing radial dilations of the gut tube and contributing to peristaltic movement of food particles down the digestive tract. In Chaetonotida, where sphlancnic circular muscles are less numerous than those of macrodasyidans, or altogether lacking, the helicoidal muscles may also contribute to closure of the pharyngeal-intestinal valve. The functional nature of the cuticular valve has yet to be determined, but if it acts in a similar fashion to the pharyngeal valve of nematodes, then back pressure from the intestine should be required to close it (Wright 1991). In *X. intermedia*, where helicoidal and intestinal circular muscles are present, both could contribute to pressurizing the intestine and closing the pharyngeal valve. In species without intestinal circular muscles (*L. squamata*), the helicoidal muscles alone may fulfill this role, perhaps accounting for their wider spacing along the intestine as opposed to the condition seen in *X. intermedia*.

Another potential function for helicoidal muscles may be in shape conservation of the alimentary tract during body movement. Muscle fiber angle relative to the longitudinal body axis may be significant in understanding these mechanics. Fiber angle generally ranged from 40-60°, with most specimens displaying 55° when in a relaxed condition. As

suggested previously (Wainwright 1988; Ruppert & Barnes 1994), helically-arranged fibers function to toughen the body-wall of animal hydrostats and prevent kinking. A fiber angle of 55° is notable because this allows the hydrostat (e.g., gastrotrich digestive tract) to shorten or lengthen over a greater range than possible with fiber angles different from 55° (Vogel 1988; Ruppert & Barnes 1994). Length change of the gastrotrich pharynx during feeding has not been documented, instead, the pharyngeal lumen expands radially (Ruppert 1991). However, length change in the gastrotrich intestine appears to be possible, especially in elongate macrodasyidan species that readily contract during escape responses or other locomotory behaviors. In such cases, the helicoidal muscles may prevent bulging or kinking of the intestine. Whether or not these muscles can contribute to shape change during locomotion (e.g., helical body twisting) remains to be determined.

# CHAPTER 6

## ORGANIZATION OF MUSCLES IN CHAETONOTIDA PAUCITUBULATINA[1]

### Introduction

Gastrotrichs of the suborder Paucitubulatina (Order Chaetonotida) are microscopic (0.1-1mm) ten-pin shaped worms with a characteristic forked caudum bearing a pair of adhesive tubes. Locomotion is generally through ciliary gliding on the substrate or in the water column. The paired adhesive tubes act as anchors for attachment and pivoting of the body, while various muscles function to bend or twist the trunk, thereby changing the direction of locomotion. Muscles are generally arranged as individual bands, though they may be intimately associated with each other to form functional muscle blocks. Examples of multiple muscle bands producing a solitary morphofunctional unit are best seen in the macrodasyidan gastrotrichs, where the ventrolateral longitudinal muscles, responsible for twisting and bending of the trunk, are made up of several long myocytes (Hochberg & Litvaitis 2001b,d). Also similar to the macrodasyidan gastrotrichs, chaetonotidan gastrotrichs possess muscles in multiple orientations including circular, longitudinal and helicoidal (Hochberg & Litvaitis 2001a).

The musculature of chaetonotidans has received limited attention since its first description by Zelinka (1889). Remane (1936) and Teuchert & Lappe (1980) contributed

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[1] Hochberg, R. & Litvaitis, M.K. 2002. Organization of muscles in Chaetonotida Paucitubulatina. Meiofauna Marina In Press.

information on some general muscle patterns and Ruppert (1991) provided important ultrastructural details on several species. Muscle organization in chaetonotidans is likely to yield relevant phylogenetic signals as it has for macrodasyidan gastrotrichs (Hochberg & Litvaitis 2001c) and provide explanatory power for understanding locomotory patterns (Banchetti & Ricci 1998; Hochberg & Litvaitis 2001b). The purpose of the present paper is to describe the muscle patterns of eleven species of Chaetonotida Paucitubulatina from two families, interpret the functions of the muscles, and place the data into a phylogenetic context.

## Material and Methods

Ten species of marine and freshwater Gastrotricha were collected from sites around New Hampshire and Maine, USA. The marine gastrotrichs, *Aspidiophorus marinus*, Remane, 1926, *Chaetonotus lacunosus* Mock, 1979, *Halichaetonotus* sp. 1 and *Xenotrichula intermedia* Remane, 1934 were collected from south Hampton Beach, New Hampshire (42° 53' 43" N, 70° 49' 13" W) in fine grain sand at the low tide level (0-2 ft). *Halichaetonotus aculifer* (Gerlach, 1953) was collected from the low intertidal of a beach in Salisbury, Maine. The freshwater gastrotrichs *Chaetonotus* cf. *murrayi*, *Chaetonotus* cf. *maximus*, *Chaetonotus* cf. *minimus*, *Chaetonotus* sp. 1 and *Lepidodermella* sp. 1 were collected from fine-grain sand and vegetation approximately 1 m deep at Sebago Lake, Maine (43° 75' 83"N , 70° 57' 34"W). All collected species with the exception of *Chaetonotus* sp. 1, *Halichaetonotus* sp. 1 and *Lepidodermella* sp. 1 were referable to their original descriptions and are considered conspecifics for the purpose of this paper. *Lepidodermella squamata* (Dujardin, 1841) was obtained from Carolina Biological Supply. Marine gastrotrichs were extracted from the sand using 7.5% magnesium

chloride (Pfannkuche & Thiel 1988), and freshwater gastrotrichs were extracted using 1% magnesium chloride.

Gastrotrichs were relaxed in $MgCl_2$ for 20 min prior to a 1hr fixation in 4% formaldehyde in 0.1M phosphate buffered saline (PBS). Fixed specimens were rinsed in 0.1M PBS, permeabilized for 1hr in 0.2% Triton X-100 in PBS, stained for 40 min with Alexa Fluor 488-phalloidin (Molecular Probes, Eugene, OR, USA), and rinsed again in PBS before mounting with Gel/Mount (Biomeda Corp.). A minimum of ten specimens of each species were viewed on a Zeiss epifluorescence microscope equipped with Spot Cooled Color digital camera (Diagnostic Instruments, Inc.). Measurements of gastrotrichs were performed with an ocular micrometer and the positions of particular organs were expressed in reference to percentage body units (total body length =100 units (U)).

## Results

Chaetonotidan muscles are generally present in circular, dorsoventral, helicoidal and longitudinal orientations. Radial muscles of the pharynx were not observed. Muscle names follow the format presented by Ruppert (1975). Muscle names used interchangeably between Xenotrichulidae and Chaetonotidae are not meant to denote homology.

### Family Xenotrichulidae

*Xenotrichula intermedia*. Circular muscles were found in splanchnic (covering the gut tube) and somatic positions (Figs. 6.1, 6.2). Anteriorly, splanchnic circular muscles were present as numerous individual rings around the pharynx (pcm). Overlying these muscles were helicoidal muscle bands (hm). Helicoidal muscles spiraled around the pharynx and formed crosses at 45° angles relative to the longitudinal body axis. The most anterior

helicoidal crosses were observed at U12, approximately halfway along the pharynx, and extended to U50 along the intestine. Longitudinal muscles were also present on the pharynx (described below) but their position (under or on) relative to the helicoidal muscles was undetermined.

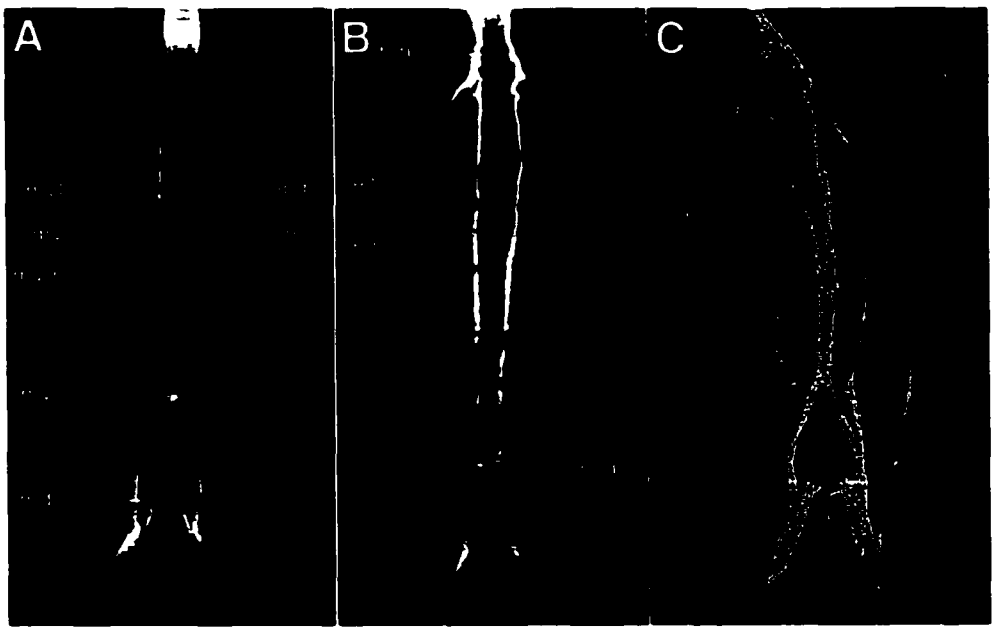


Figure 6.1. Whole-mounts of *Xenotrichula intermedia* stained with Alexa 488-phalloidin. A, ventral view, trunk region. B, dorsal view, trunk region. C, ventrolateral view, trunk region. Scale bars = 30 μm. hm – helicoidal muscle; md – musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvc – cross-over region of musculi ventrales; mvl – musculi ventrolaterales; mvm – musculi ventromediales; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; scm – incomplete somatic circular muscles; scmi – ventral insertion points of incomplete somatic circular muscles.

In the trunk region, six muscle orientations were present: complete circular, incomplete circular, dorsoventral, diagonal, helicoidal and longitudinal (Fig. 6.2). Circular muscles, *sensu strictu*, were present as individual rings around the anterior third

of the intestine (cicm). The muscles were sparse (< 5), thin (1 μm) and spread apart. Posteriorly, two types of incomplete circular muscles were present. These muscles differed from dorsoventral muscles *sensu strictu* in that the former were dorsally continuous (made dorsal arches over the intestine) but incomplete ventrally. The first type of incomplete circular muscle was present in a splanchnic position (Fig. 6.2, icm). These muscles made a dorsal arch over the intestine and inserted on the ventral bodywall on either side of the intestine (icmi). Incomplete somatic circular muscles existed as dorsolateral extensions of the splanchnic component (scm), with insertion points on the ventrolateral body wall (scmi). These muscles were evident in the anterior and posterior region of the trunk. In the middle of the trunk, the incomplete somatic circular muscles were replaced by dorsoventral muscles *sensu strictu* (dvm). These muscles had dorsal and ventral insertions. The number of somatic circular muscles and dorsoventral muscles was not determined.

Five pairs of bilateral longitudinal muscles extended from a region on the pharynx to the caudum: the musculi dorsales (md), musculi ventrales (mv), musculi ventromediales (mvm), musculi ventrolaterales (mvl), and musculi laterales (ml). The musculi dorsales inserted anteriorly at the top of the pharynx and posteriorly in the furca (Fig. 6.2). Approximately 25 μm posterior to the pharyngeointestinal junction the musculi dorsales branched producing the Rückenhautmuskel (after Zelinka 1889). The paired Rückenhautmuskel (R1) bowed laterally and was often in contact with the dorsolateral body wall of the trunk. When eggs were present, the muscle wrapped tightly around the dorsolateral margin of the eggs. Posteriorly, the Rückenhautmuskel inserted into the base of the caudum, independent of the musculi dorsales. At approximately U80,

the musculi dorsales branched a second time, producing two thin medial branches that crossed each other at the midline and entered the contralateral furcal branch (Fig. 6.2).

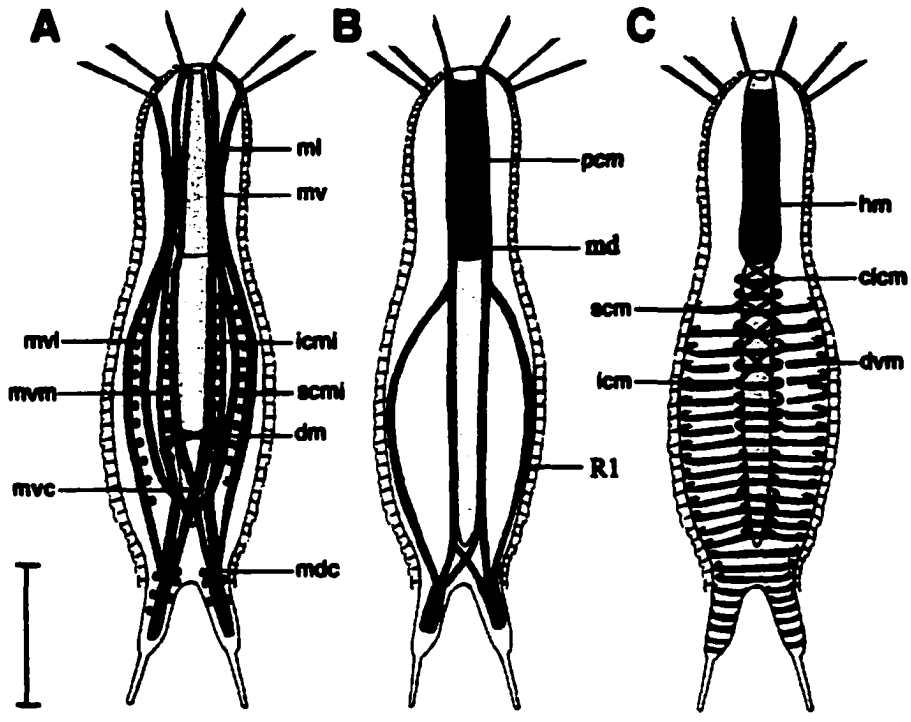


Figure 6.2. Schematic arrangement of muscles in *Xenotrichula intermedia*. A, ventral view showing longitudinal muscles and insertion points of incomplete circular muscles. B, dorsal view showing longitudinal muscles and circular muscles of pharynx. C, dorsal view, showing helicoidal muscles and various circular muscles. Scale bar = 30 μm. cicm – complete circular muscles of intestine; dm – diagonal muscle branch of mvm; dvm – dorsoventral muscle; hm – helicoidal muscle; icm – incomplete circular muscle of intestine; icmi – ventral insertion points of incomplete circular muscles of intestine; md – musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvc – cross-over region of musculi ventrales; mvl – musculi ventrolaterales; mvm – musculi ventromediales; pcm – pharyngeal circular muscle; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; scm – incomplete somatic circular muscles; scmi – ventral insertion points of incomplete somatic circular muscles.

Four pairs of longitudinal muscles were located in the ventral and lateral regions of the body (Fig. 6.2). Three of these muscle sets thickened (increased diameter) caudally. The musculi ventrales arose from the anterior portion of the pharynx and extended caudally. At ca. U80, the musculi ventrales crossed each other at the midline and inserted into the contralateral basis of the furca. A second pair of muscles (musculi ventromediales) arose from the lateral wall of the pharynx and remained in close proximity to the musculi ventrales for most of the length of the body (Fig. 6.2). Slightly anterior to the region where the musculi ventrales crossed each other, two thin diagonal muscle branches (dm) arose from the musculi ventromediales, crossed the body midline, and remained in close contact with the contralateral musculi ventromediales along its posterior length. The musculi ventrolaterales arose laterally from the pharynx wall and ran posteriorly where it inserted on the musculi ventrales posterior to the cross over. The most lateral muscle pair (musculi laterales) was the only pair not to be in contact with the gut tube along most of its length (Fig. 6.2). Anteriorly, the musculi laterales inserted on the bodywall lateral to the pharynx. Posteriorly, the muscles bent ventromedially around the pharyngeointestinal junction and then flared laterally to follow the shape of the body until the caudal region where they inserted in the furca.

Family Chaetonotidae

Muscle patterns of all examined species were similar and their general arrangement is described here. Individual differences are noted for each species below.

Circular muscles are present as individual rings around the pharynx only. Overlying the circular rings on the posterior portion of the pharynx were helicoidal muscles. These muscles crossed one another to form 45-50° angles and spiraled caudally

until ca. U50. Helicoidal muscles lay external of the musculi ventrales and musculi dorsales (e.g., Fig. 6.3).

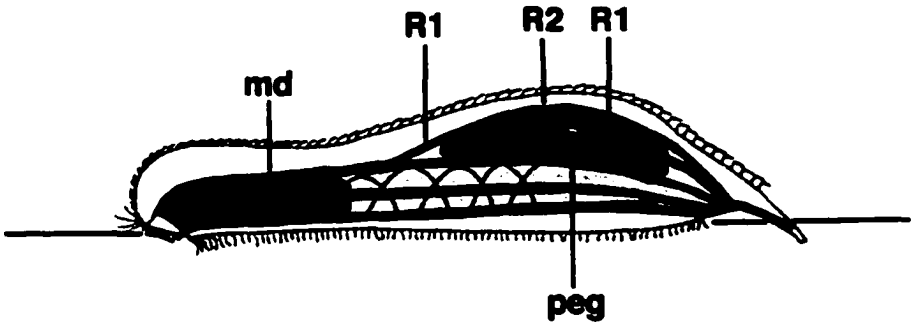


Figure 6.3. Schematic arrangement of muscles in *Aspidiophorus marinus*, lateral view. md – musculi dorsales; peg – parthenogenic egg; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; R2 – dorsomedial branch of Rückenhautmuskel.

Four pairs of longitudinal muscles extended the length of the body: the musculi ventrales, musculi ventrolaterales, musculi laterales, and musculi dorsales (e.g., Fig. 6.5). A fifth pair of muscles (Rückenhautmuskel after Zelinka 1889) was found only in the trunk. The musculi ventrales (ca. 2 μm thick) lay along the midline of the gut tube, inserting anteriorly on the pharynx and posteriorly in the caudal furca. This muscle pair thickened up to 5μm in diameter before entering each ramus of the furca. The musculi ventrolaterales (1-2 μm) inserted on the lateral body wall in the head region, bent inward around the pharygeointestinal junction, and joined the musculi laterales in the posterior region of the body (ca. U90). The musculi laterales, often the thickest muscles in the body (3-6 μm) inserted anteriorly on the lateral wall of the pharynx and followed the shape of the body caudally, bending outward in the trunk region. Around U85, the

musculi laterales bent ventrally and joined the musculi ventrolaterales to enter the caudal furca. The musculi dorsales inserted anteriorly at the top of the pharynx and ran caudally along the intestine, finally inserting ventrally behind the anus close to the body midline. Posterior to the pharyngeointestinal junction, the musculi dorsales bifurcated to form the Rückenhautmuskel (R1). The Rückenhautmuskel was not bound to the intestine by helicoidal muscles as were the musculi dorsales. Instead, it was often external to a maturing egg that lay dorsal on the intestine. The Rückenhautmuskel bifurcated to produce two branches, a dorsolateral branch (R1) and a dorsomedial branch (R2). Both muscles bowed reunited separately with the musculi ventrales around U75 (Fig. 6.3).

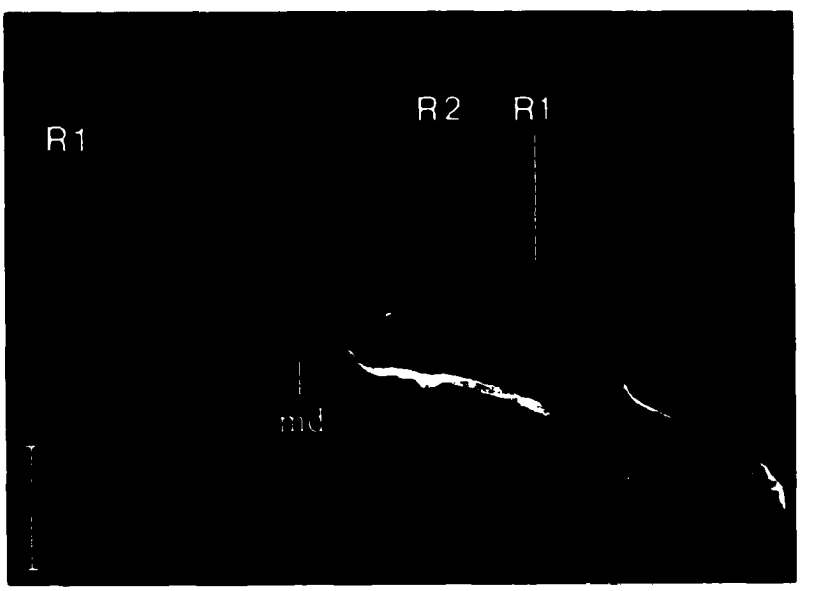


Figure 6.4. Whole-mount of *Aspidiophorus marinus* stained with Alexa 488-phalloidin. Dorsolateral view of trunk region. md – musculi dorsales; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; R2 – dorsomedial branch of Rückenhautmuskel.

A bilateral pair of dorsoventral muscles was located at the posterior end just before the caudal furca. This muscle pair originated from the musculi dorsales and connected to the musculi ventrales. A single pair of muscle 'spikes' (pms) was present at the base of the body just before the caudal furca. These spikes were directed anteriorly and appeared to branch off the musculi laterales.

*Aspidiophorus marinus* (Figs. 6.3-6.5). Anteriorly, the musculi ventrolaterales remained parallel to the pharynx and inserted close to the mouth. The Rückenhautmuskel bifurcated around U45 to produce R1 and R2. Branch R1 inserted around U80 on or near the musculi ventrales. Branch R2 inserted around U85-90 on or near the musculi ventrales. Posterior muscle spikes and dorsoventral muscles were absent.

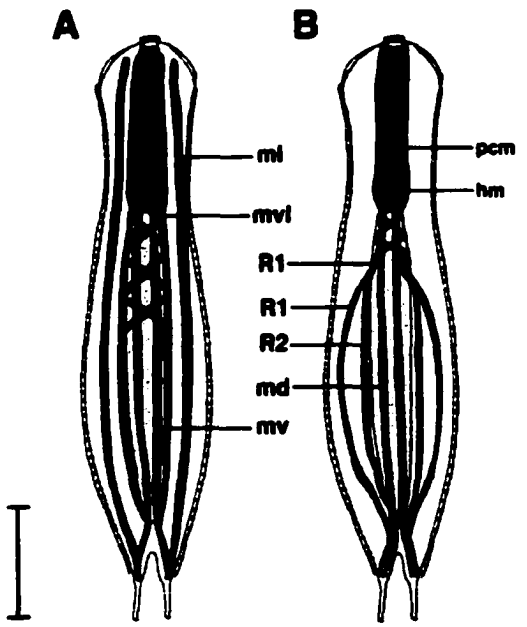


Figure 6.5. Schematic arrangement of muscles in *Aspidiophorus marinus*. A, ventral view. B, dorsal view. Scale bar = 20 μm. hm – helicoidal muscle; md – musculi dorsales; mdc – connection to the musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvl – musculi ventrolaterales; mvm – musculi ventromediales; pcm – pharyngeal circular muscle; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; R2 – dorsomedial branch of Rückenhautmuskel.

*Chaetonotus lacunosus*. *Chaetonotus* cf. *murrayi*, *Chaetonotus* cf. *maximus*, *Chaetonotus* cf. *minimus*, *Chaetonotus* sp. 1 (Figs. 6.6, 6.7). The Rückenhautmuskel bifurcated into branches R and R1 approximately 20-30 um posterior to the origin of the Rückenhautmuskel. Branches R1 and R2 remained separate for their length. In the posterior body region both muscles bent ventrally and united with the musculi ventrales separately. Posterior muscle spikes were not observed in *Chaetonotus* cf. *maximus*.

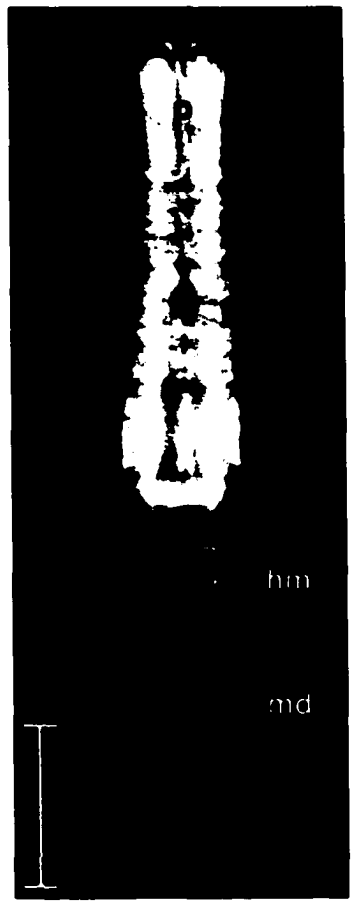


Figure 6.6. Whole-mount of *Chaetonotus lacunosus* stained with Alexa 488-phalloidin. Dorsal view of anterior end. Scale bar = 15 μm. hm – helicoidal muscle; md – musculi dorsales.

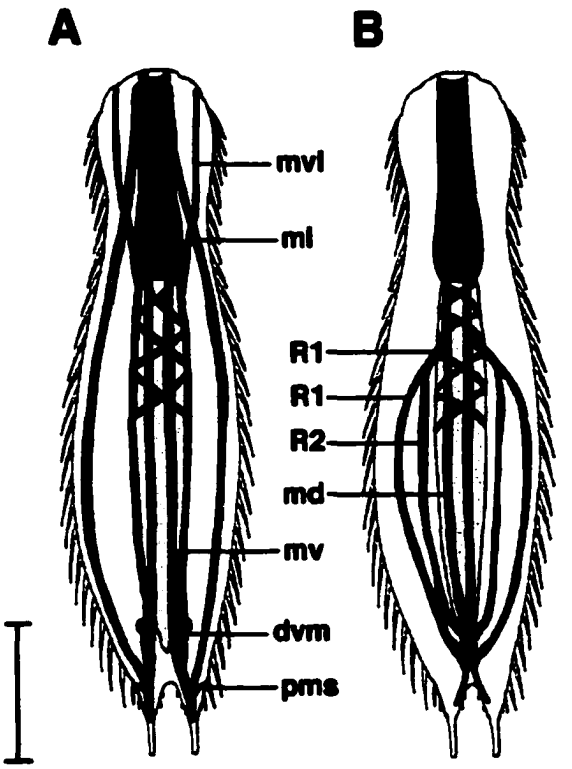


Figure 6.7. Schematic arrangement of muscles in several species of *Chaetonotus*. A, ventral view. B, dorsal view. Scale bar = 20 µm. dvm – dorsoventral muscle; md – musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvl – musculi ventrolaterales; pms – posterior muscle spikes; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally; R2 – dorsomedial branch of Rückenhautmuskel (R1).

*Halichaetonotus aculifer*, *Halichaetonotus* sp. 1 (Fig. 6.8). The musculi laterales inserted anteriorly on the lateral body wall of the head. In *H. aculifer*, the musculi laterales produced one short branch at ca. U40 where it inserted on the lateral body wall. In *Halichaetonotus* sp. 1, numerous short muscle branches were directed toward the lateral body wall. In both species, the musculi laterales branched in the caudal region prior to insertion; the main branch inserted on the posterior bodywall, and the medial branch joined the musculi ventrales. Also in both species, the Rückenhautmuskel reconnected

with the musculi dorsales prior to its insertion in the caudal region. Additionally, the posterior dorsoventral muscles wrapped around the musculi laterales. Absent from both species was the bifurcation of the Rückenhautmuskel (R2) and the posterior muscle spikes of the musculi laterales. In addition, *H. aculifer* lacked the musculi ventrolaterales.

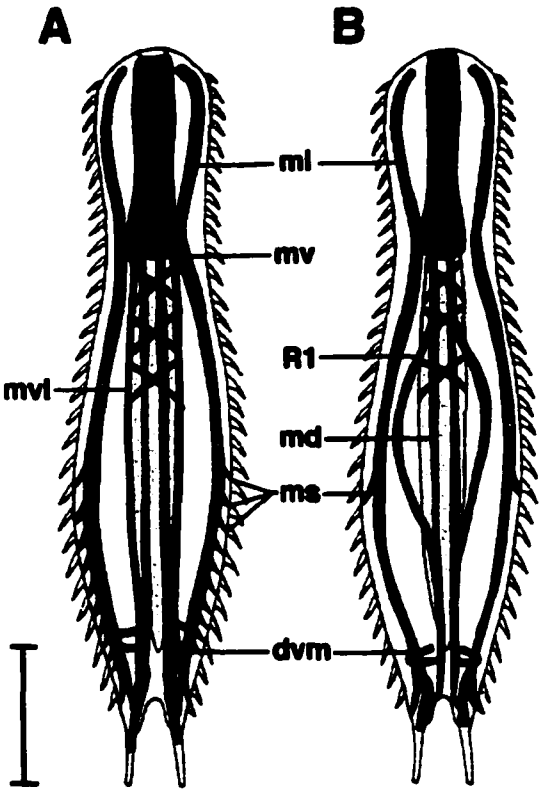


Figure 6.8. Schematic arrangement of muscles in two species of *Halichaetonotus*. A, ventral view. B, dorsal view. Note that mvl and multiple muscle spikes are only present in *Halichaetonotus* sp. 1. Scale bar = 25 μm. dvm – dorsoventral muscle; md – musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvl – musculi ventrolaterales; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally.

*Lepidodermella squamata*, *Lepidodermella* sp. 1 (Figs. 6.9, 6.10, 6.11). The musculi laterales inserted close to the mouth, followed the shape of the pharynx posteriorly, and bent laterally to follow the shape of the body to the caudum. The musculi ventrolaterales inserted lateral to the mouth on the bodywall, bent ventromedially toward the pharyngeointestinal junction, and followed the musculi ventrales into the caudal furca. Muscles spikes and the bifurcation of the Rückenhautmuskel (R2) were only present in *Lepidodermella* species 1.

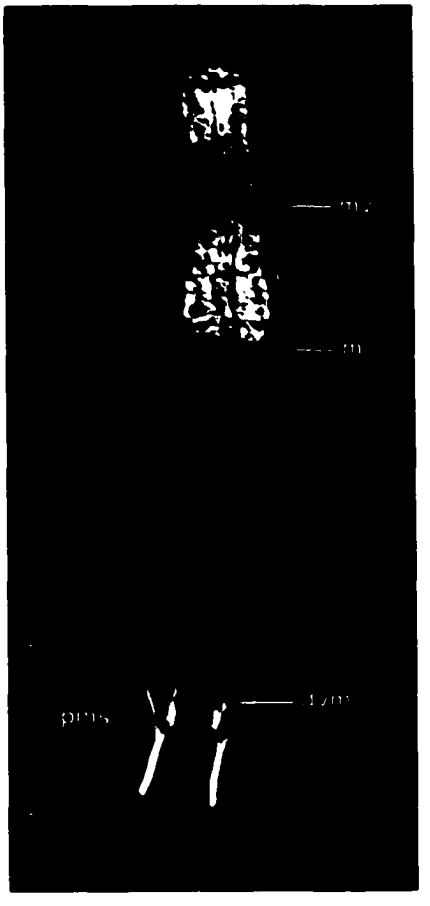

Figure 6.9. Whole-mount of *Lepidodermella* sp. 1 stained with Alexa 488-phalloidin. Dorsal view. Scale bar = 40 µm. dvm – dorsoventral muscle; ml – musculi laterales; mvl – musculi ventrolaterales; pms – posterior muscle spikes.

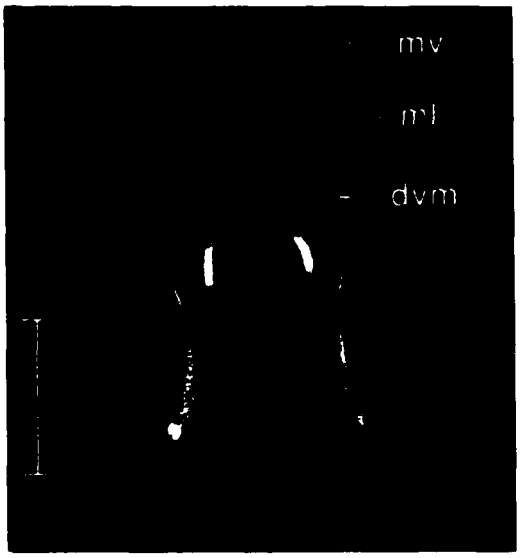


Figure 6.10. Whole-mount of *Lepidodermella squamata* stained with Alexa 488-phalloidin. Ventral view of furcal region. Scale bar = 10 μm. dvm – dorsoventral muscle; ml – musculi laterales; mvl – musculi ventrolaterales.

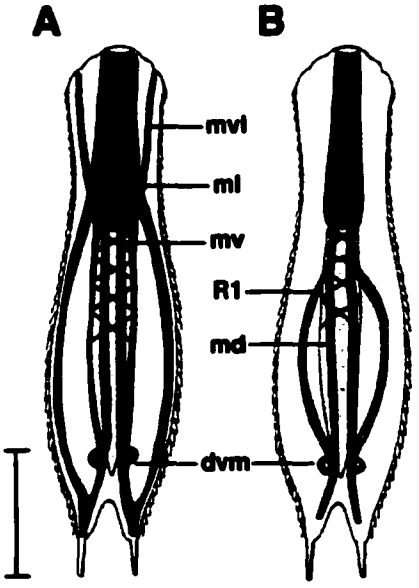


Figure 6.11. Schematic arrangement of muscles in two species of *Lepidodermella*. A, ventral view. B, dorsal view. The bifurcation of the Rückenhautmuskel and posterior muscle spikes are not pictured (only present in *Lepidodermella* sp. 1). Scale bar = 40 μm. dvm – dorsoventral muscle; md – musculi dorsales; ml – musculi laterales; mv – musculi ventrales; mvl – musculi ventrolaterales; R1 – Rückenhautmuskel, first branch off md that continues dorsolaterally.

## Discussion

Chaetonotidan gastrotrichs represent some of the smallest known animals from aquatic habitats, and their stout body shape and rigid cuticle are likely adaptations to the interstitial conditions in which they evolved. Freshwater gastrotrichs are undoubtedly derived from marine forms (see Kisielewski 1990). However, despite obvious differences in the interstices of marine, coastal vs. inland, lentic environments (e.g., water movement, types of biota, composition of sand grains), gastrotrich body form of marine and freshwater species is generally similar (with few exceptions, e.g., *Kijanebalola*, see Ruppert 1988). Within the interstices of marine sands, the size and availability of pore space for animal growth and movement is limited by the size and shape of the sand grains and how well they are sorted within a particular locale. The same holds true for freshwater sediments, though many freshwater gastrotrichs are not limited to these pore spaces and often venture into the water column or onto benthic vegetation.

Locomotion in nearly all chaetonotidan gastrotrichs is mainly via the ventral cilia, whether in the form of longitudinal rows, small tufts, or as 'hypotrichous' cirri (Hummon 1974). Locomotory patterns include slow and fast substrate gliding and spiral-like swimming (Bancetti & Ricci 1998). Muscles are mainly used for feeding (pharyngeal pumping), changing directions during ciliary gliding, or to pivot a gastrotrich about its caudal attachment to the substrate. This limited range of known gastrotrich movement patterns can be correlated with the known patterns of muscles to infer function.

Muscles of chaetonotidans are arranged in antagonistic groups (complete circular bands and longitudinals, incomplete circular bands and longitudinals, dorsoventral bands and longitudinals, and helicoidal bands and longitudinals) and are distributed along the

longitudinal body axis. Splanchnic circular muscles are present on the pharynx of all examined species of Chaetonotidae and *Xenotrichula intermedia*, but are present only on the intestine of *X. intermedia* (also known for *X. carolinensis* Ruppert, 1979 (Travis 1983) and *Heteroxenotrichula transatlantica* Ruppert, 1975). Incomplete splanchnic circular muscles are present in *X. intermedia*, and a more derived form occurs in *Draculicitera tessalata* (Hochberg & Litvaitis 2001e). Incomplete somatic circular muscles and dorsoventral muscles occur in the trunk region of *X. intermedia* and other xenotrichulids (see Ruppert 1975 for *H. transatlantica* and others; Hochberg & Litvaitis 2001e for *D. tessalata*). Helicoidal muscles are well known from macrodasyidan gastrotrichs (Hochberg & Litvaitis 2001d), and are present in all ten species of Chaetonotida (also *D. tessalata*, Hochberg & Litvaitis 2001e). Longitudinal muscles are arranged bilaterally and constitute the longest muscles in the body of all gastrotrichs.

As in the macrodasyidan gastrotrichs, the circular muscles on the pharynx of chaetonotidans are likely to serve as antagonists to pharyngeal pumping, quickly restoring the tri-radiate lumen after imbibing water during feeding. Overlying these muscles are the longitudinal muscles and helicoidal bands. The likely function of the helicoidal bands on the pharynx, as on the intestine, is to antagonize the longitudinal muscles, provided the longitudinal muscles lie beneath the helicoidal bands as they do in *D. tessalata* and various macrodasyidans (Hochberg & Litvaitis 2001d). In several species, the layering of the helicoidal bands relative to various longitudinal muscles was difficult to visualize accurately. Helicoidal muscles may also function to pressurize the intestine for purposes of defecation.

Complete circular muscles are generally absent from the trunk region of most chaetonotidans; the only known exceptions are species of *Xenotrichula* (Fig. 6.2; also *X. carolinensis*, Travis 1983). Most circular muscles are incomplete and present in two positions: splanchnic and somatic. In *X. intermedia*, the dorsal somatic component is formed via lateral branches of the splanchnic circular muscles. The incomplete circular bands lining the intestine are complete dorsally and incomplete ventrally (Fig. 6.2). Such an unusual condition is similar to the incomplete circular bands of the rotifer *Philodina* (Hochberg & Litvaitis 2000a), where the muscles presumably function to compress the body in the dorsoventral plane and antagonize longitudinal muscle contraction. Dorsoventral muscles may also cause compression and function in the movement of parthenogenic eggs through the body wall and perhaps pressurize the gut tube for purposes of defecation. Curiously, most chaetonotidan gastrotrichs lack splanchnic circular muscles and all lack pharyngeal pores (present in Macrodasyida). If the function of pharyngeal pores is to release water imbibed during feeding (Ruppert 1991), thereby prevent intestinal 'bloating', one would expect gastrotrichs lacking pharyngeal pores to possess splanchnic circular muscles that can antagonize the radial expansion of the gut. Why chaetonotidan gastrotrichs generally lack a well-developed splanchnic circular musculature is unknown, but it may be functionally correlated to the presence of a sculpted cuticle that can also resist expansion caused by hydrostatic pressure (Travis 1983).

In general, four sets of longitudinal muscles (musculi dorsales, musculi laterales, musculi ventrolaterales, musculi ventrales) are present in all chaetonotidans, and their primary function is to bend the body in lateral and dorsoventral planes and variations

thereof. Such movements are undoubtedly useful for weaving through the three-dimensional pore system of the interstitial environment. Additional muscles may serve functions other than locomotion. The main branch off the musculi dorsales, the Rückenhautmuskel (R1), is found in all chaetonotidans examined. This muscle pair forms a dorsal arch over developing parthenogenic and/or mictic eggs. Most species of Chaetonotidae possess a bifurcation of the Rückenhautmuskel to form two branches, creating a dorsolateral (R1) and dorsomedial (R2) arch over parthenogenic eggs. The function of both these muscle sets is presumably to maintain the position of the relatively large egg during its development, and then force it ventrally around the intestine and out the ventral body wall. In *Halichaetonotus aculifer* and *Lepidodermella squamata*, there was no bifurcation of the Rückenhautmuskel.

Small branches off the musculi laterales were common among members of the Chaetonotidae. In *H. aculifer* and *Halichaetonotus* sp. 1, muscle branches from the musculi laterales connected to the lateral body wall in the trunk region. In species of *Chaetonotus*, small muscle spikes connected muscles in the caudal furca to the lateral body wall. The function of these small muscle branches remains unknown, but they may function to handle additional tension during flexion of the musculi laterales.

Phylogenetic Considerations

Species of Chaetonotida examined here possess a stereotypical muscle organization that consists of circular muscles on the pharynx, helicoidal muscles on pharynx and intestine, four pairs of longitudinal muscles in dorsal, lateral, ventrolateral and ventral positions, and a paired Rückenhautmuskel. The only significant deviations from this stereotype occur in the presence of circular/dorsoventral muscles in the trunk

region of *X. intermedia* and the relative position, orientation and branching of the longitudinal muscles among Chaetonotidae.

Dorsoventral muscles occur in a variety of species examined here and elsewhere (Ruppert 1975, 1979; Hochberg & Litvaitis 2001e). In *X. intermedia*, dorsoventral muscles occur in the middle of the trunk region between anterior and posterior sets of incomplete somatic circular muscles. In *Draculicitera tessalata*, muscles in identical positions to those of *X. intermedia* (splanchnic and somatic circulars) are all dorsoventrally oriented. The anatomical transition from incomplete somatic circular muscle to dorsoventral muscle in *X. intermedia*, and identical position of dorsoventral muscles in *D. tessalata* with the somatic circular muscles of *X. intermedia*, suggests that dorsoventral muscles are derived from incomplete somatic circular muscles. This scenario fits well with two hypotheses (Ruppert 1975; Hochberg and Litvaitis 2001e) that suggest recent species reflect a hypothetical evolutionary transformation from a plesiomorphic state (some incomplete circular muscles, some dorsoventral muscles, *X. intermedia*) to a derived state (all dorsoventral muscles, *D. tessalata*). A more thorough sampling of species from this family may reveal additional species with a more plesiomorphic muscle pattern (all incomplete circular muscles and no dorsoventral muscles) than present in *X. intermedia*. In species of Chaetonotidae, a single pair of thin dorsoventral muscles is present only at the posterior end, forming connections between the musculi dorsales and musculi ventrales. In species of *Chaetonotus* and *Lepidodermella*, the dorsoventral muscles enwrap the musculi ventrolaterales. In species of *Halichaetonotus*, the dorsoventral muscles also wrap around the musculi laterales. The function of this tenuous connection is unknown, as is the functional difference of

enclosing different muscles. Based on the position and insertion of these dorsoventral muscles, they are probably not homologous to those of Xenotrichulidae.

The specialized dorsal egg-stabilizing muscle, the Rückenhautmuskel, is probably homologous among all chaetonotidans. Interestingly, most species of Chaetonotidae possess a branched Rückenhautmuskel. Both species of *Halichaetonotus* and also *Lepidodermella squamata* apparently lack this bifurcation, which may indicate a close evolutionary relationship. The simplest evolutionary scenario would predict the evolution of the Rückenhautmuskel only once in the Paucitubulatina, presumably as an adaptation to stabilize developing eggs that are disproportionately large in all chaetonotidans. Whether the bifurcation of the Rückenhautmuskel into two units is apomorphic to the Chaetonotidae or represents a plesiomorphy (lost in Xenotrichulidae) is unknown.

Ruppert (1975, p. 193) hypothesized the basic organization for the musculature of the Paucitubulatina as follows: circular muscles on the pharynx, weakly developed somatic circular muscles in the trunk region, bilaterally arranged dorsoventral muscles, and three to fours pairs of longitudinal muscles that extend the length of the body. Information derived from the species examined in this study lends itself to an emendation of the basic organization of the Paucitubulatina. The muscle arrangement of the ground pattern is proposed to consist of circular muscles on the pharynx and part of the intestine, incomplete somatic and splanchnic circular muscles in the trunk, dorsoventral muscles in the trunk, four to five pairs of longitudinal muscles, and a specialized pair of dorsal muscles (Rückenhautmuskel). By extension, the Xenotrichulidae is an early evolutionary line within the suborder based on the retention of the following plesiomorphies (shared with Macrodasyida): circular muscles on pharynx and intestine and somatic circular

muscles in trunk. Several other lines of research (pharynx ultrastructure, Ruppert 1982; body wall ultrastructure Travis 1983; general morphology, Hochberg & Litvaitis 2000c) substantiate this position for the Xenotrichulidae. The absence of dorsoventral muscles *sensu strictu*, from any macrodasyidans and members of the Chaetonotidae, suggests they are apomorphic to the Xenotrichulidae (see also Ruppert 1975).

The position of the Chaetonotidae within the Paucitubulatina is more tenuous. Two alternative hypotheses include the family as a primitive but paraphyletic clade (Wirz et al. 1999) or a derived grouping that is paraphyletic (Hochberg & Litvaitis 2000b). With more than 200 species distributed among nine genera from marine and freshwater habitats, the family is undoubtedly the largest in the Gastrotricha. Evolutionary relationships within the Chaetonotidae are consequently not well established (Hochberg & Litvaitis 2000c). Ruppert (1975, p. 205) suggests that *Musellifer* and *Polymerurus* are probably early divergent taxa, and species from these genera may therefore retain a greater number of muscular plesiomorphies. Further evidence suggests that species of *Aspidiophorus*, *Chaetonotus* and *Lepidodermella* sp. 1 may be closely related because they possess a branched Rückenhautmuskel , and by extension, *Halichaetonotus* and *L. squamata* may be more primitive (never possessed R2) or derived (independent evolutionary loss of R2). The absence of the musculi ventrolaterales from *H. aculifer* (but present in *Halichaetonotus* sp. 1) is interesting and may point toward a more derived condition of the species.

Concluding Remarks

This brief survey of muscle patterns in chaetonotidan gastrotrichs suggests that there are differences between members of the Xenotrichulidae and Chaetonotidae, and

that these differences (and similarities) may be of phylogenetic value. However, evolutionary loss or addition of muscles remains difficult to assess without a more thorough sampling of additional species of Xenotrichulidae, and especially species of *Musellifer*, *Polymerurus*, and the aberrant *Neodasys*. That *Neodasys* is an anomalous genus of Chaetonotida is well-known (Tyler et al. 1980; Ruppert 1991), and its importance for a better understanding of gastrotrich phylogeny can not be overstated. While our knowledge of the muscular system of *Neodasys* is limited (Ruppert 1975, 1991; Teuchert & Lappe 1980; Ruppert & Travis 1983) evidence suggests that members of the genus possess plesiomorphic characteristics (circular muscles on pharynx, several blocks of longitudinal muscles) and derived characteristics (absence of somatic and intestinal circular muscles). A remnant of the complete splanchnic circular musculature may remain as a sphincter around the hindgut in *N. chaetonotoideus* (Ruppert 1975, pp. 209). Whole-mount observations of *Neodasys* could verify the presence of muscles (and more importantly, their orientations) difficult to view with electron microscopy (e.g., helicoidal muscles, intestinal and somatic circular muscles that are spaced apart). With additional sampling of species, insight into the functional role of various muscle groups could be elucidated, as would knowledge of evolutionary transformations within their respective families.

# CHAPTER 7

## THE MUSCULATURE OF *DRACULICITERIA TESSALATA* (GASTROTRICHA, CHAETONOTIDA)[1]

### Introduction

Understanding the morphology and evolution of gastrotrichs is complicated by their minute size and a general lack of detailed information on their internal anatomy. The family Xenotrichulidae (Order Chaetonotida), however, represents a unique case among gastrotrichs in that detailed morphological data exists on all its inclusive genera (Ruppert, 1975, 1979). Light microscopical sections have revealed morphological synapomorphies for the family, and provided important information on their evolution.

In his monograph of the Xenotrichulidae, Ruppert (1975, 1979) erected two subfamilies to reflect the clearly divergent nature of the family's three genera. *Xenotrichula* and *Heteroxenotrichula* comprise the subfamily Xenotrichulinae, a monophyletic lineage based on cuticle structure and reproductive anatomy (Ruppert, 1975). The monospecific *Draculiciteria* was placed into the Xenotrichulidae by Hummon (1974) and was later moved to the subfamily Draculiciterinae by Ruppert (1975) because of differences in ciliary patterns, scale construction, reproductive anatomy and muscular system.

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1. Hochberg, R. & Litvaitis, M.K. 2001. The musculature of *Draculiciteria tesslata* (Gastrotricha, Chaetonotida). Hydrobiologia 452: 155-161.

The muscular system has provided important information on gastrotrich evolution, from the gross anatomical level (Ruppert, 1975, 1979; Hochberg & Litvaitis 2001d) to the ultrastructural level (Travis, 1983; Ruppert, 1982, 1991). Although the muscular systemof chaetonotidans (Zelinka 1889; Remane 1935-1936) has received comparatively less attention than the macrodasyidan gastrotrichs (Teuchert, 1974, 1977; Ruppert, 1991, and references therein), Ruppert (1975) has provided a foundation upon which to build more detailed evolutionary studies within the order.

In the present study, we report on additional aspects of the musculature of *Draculiciteria tessalata* using a fluorescent phalloidin stain. Muscle patterns are described and potential evolutionary origins of specific muscle sets are hypothesized.

## Materials and Methods

*Draculiciteria tessalata* (Renaud-Mornant, 1968) was collected from south Hampton Beach, New Hampshire (70° 49' 13" W, 42° 53' 43" N) at the low tide level (0-2 ft) in medium to fine grain sand. Gastrotrichs were extracted from the sediment using an anesthetization/decantation technique with 7.5% magnesium chloride (Pfannkuche & Thiel, 1988). Fixation and staining protocols using Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR) followed Hochberg and Litvaitis (2000). Specimens were viewed on a Zeiss epifluorescence microscope equipped with Spot Cooled Color digital camera (Diagnostic Instruments, Inc.). Measurements of gastrotrichs were performed with an ocular micrometer and the positions of muscles are expressed in reference to percentage body units (total body length =100 units (U)).

## Results

Muscle descriptions utilize earlier terminology established by Ruppert (1975). Fifteen specimens of adult *Draculicteria tessalata* (Fig. 7.1; average body length 210 μm) were examined. The musculature of *D. tessalata* consisted of muscles in circular, longitudinal, helicoidal, and dorso-ventral orientations (Figs. 7.1-7.5).

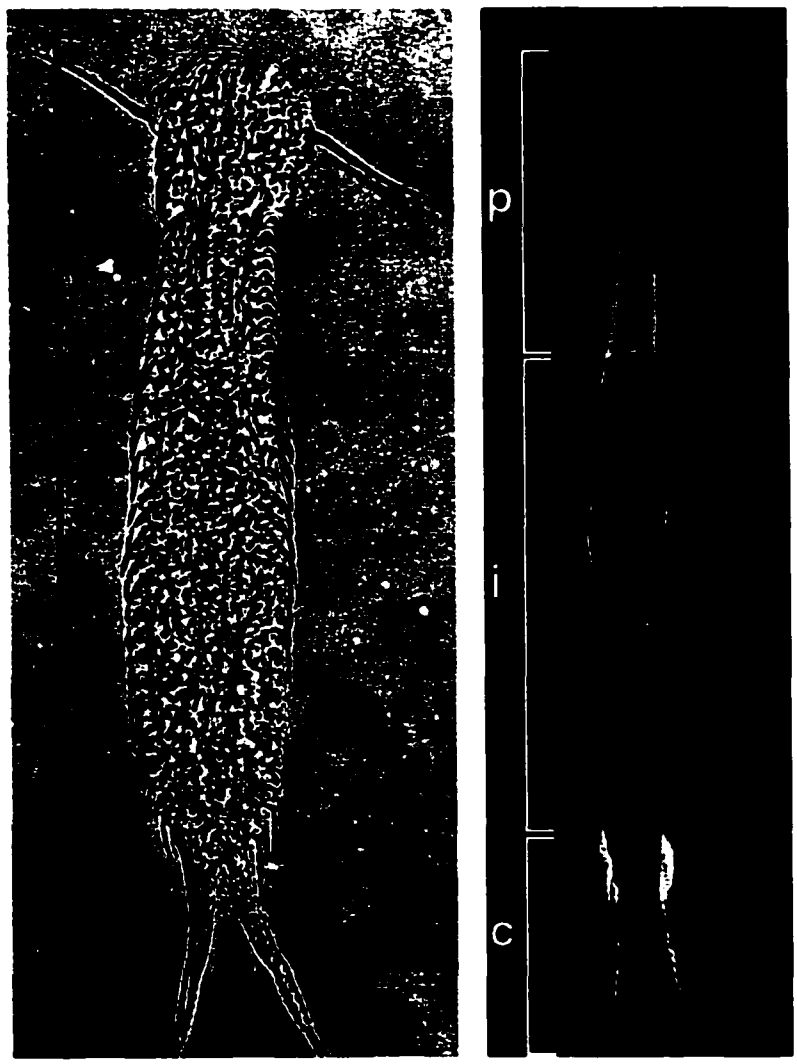



Figure 7.1. *Draculiciteria tessalata*. Left: light micrograph. Right: phalloidin stained whole mount. p – pharynx region, i – intestine/trunk region, c – caudal furca, ci – cirri extending from pleurion. Scale bar is 50 μm.

Circular muscles were present as individual bands that lined the pharynx from the mouth rim to the pharyngeal-intestinal junction (Fig. 7.1). Circular muscles were approximately 1-2 μm in diameter and spaced evenly along the pharynx. Forty circular bands were counted on the pharynx (65 μm length) of a single specimen (200 μm body length). No circular muscles were observed posterior to the pharyngeal-intestinal junction.

Helicoidal muscles lined the entire pharynx and approximately half the length of the intestine (Figs. 7.2, 7.5). These muscles spiraled around the gut tube, forming crosses with angles of approximately 50-55° to the longitudinal body axis. On the pharynx, helicoidal muscles appeared to be external of the circular muscles, but it was not determined if they were also external of the longitudinal muscles. On the intestine, helicoidal muscles encircled one pair of longitudinal muscles (musculi ventrales).

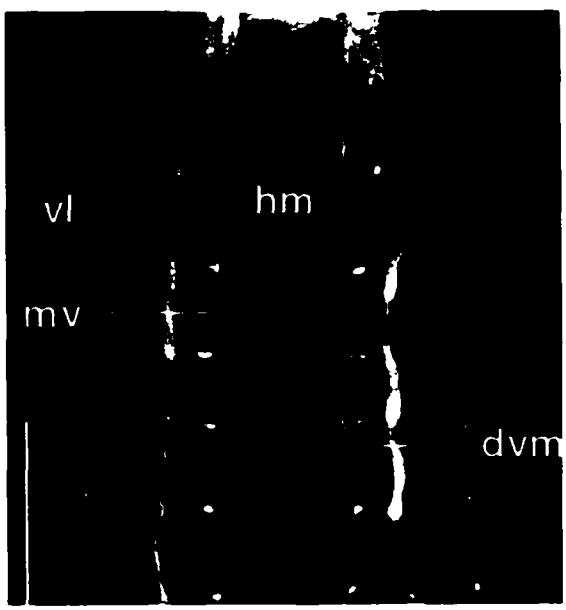



Figure 7.2. Ventral view of anterior trunk region directly below pharyngeal-intestinal junction. dvm – medial dorsoventral muscle, hm – helicoidal muscles, mv – musculi ventrales, vl – musculi ventrolaterales. Scale bar is 15 μm.

Longitudinal muscles were found in dorsal, lateral and ventral positions relative to the alimentary tract (Figs. 7.1-7.4). Most longitudinal muscles were 1-2 μm wide. Dorsally, two thin longitudinal muscles (musculi dorsales) spanned the dorsolateral length of the entire gut tube (Fig. 7.4). This muscle pair inserted close to the mouth rim, remained in contact with the pharynx and anterior portion of the intestine, and flared dorsally around U35 until bending ventrally and joining a pair of ventral muscles around U85. It was not adequately determined if the musculi dorsales inserted on the musculi ventrales (Fig. 7.5) or musculi ventrolaterales, or merely remained in close proximity to them as they entered the caudal furca.

Ventrally and laterally, there were four pair of longitudinal muscles that extended most of the length of *D. tessalata* (Figs. 7.2-7.4). The most ventral muscle pair (musculi ventrales) was located ventral to the gut tube and remained in contact with it along its entirety. These muscles inserted anteriorly at the mouth rim and posteriorly crossed-over one another at U68-U70 before inserting into each ramus of the caudal furca. Slightly lateral to the musculi ventrales were the musculi ventrolaterales. This muscle pair also inserted anteriorly at the mouth rim and posteriorly joined the musculi ventrales at U90 before inserting into each branch of the caudal furca. A third pair of longitudinal muscles (musculi paralaterales) was located lateral to the musculi ventrolaterales and abutted the former muscles for most of their length. Musculi paralaterales were extremely thin (< 1 μm), inserted anteriorly on the mouth tube (obscured by musculi ventrolaterales) and posteriorly entered each branch of the caudal ramus. At approximately U55, the muscle pair produced a thin anteriorly-directed branch that appeared to supply the most lateral muscle pair (musculi laterales). The musculi laterales began anteriorly around the lateral

head appendages (pleurions with lateral cirri) and extended posteriorly into the caudal furca, joining the musculi ventrales, musculi ventrolaterales, and musculi paralaterales. In the head and trunk region, the musculi laterales flared laterally and never contacted the gut tube (Figs. 7.1, 7.5).

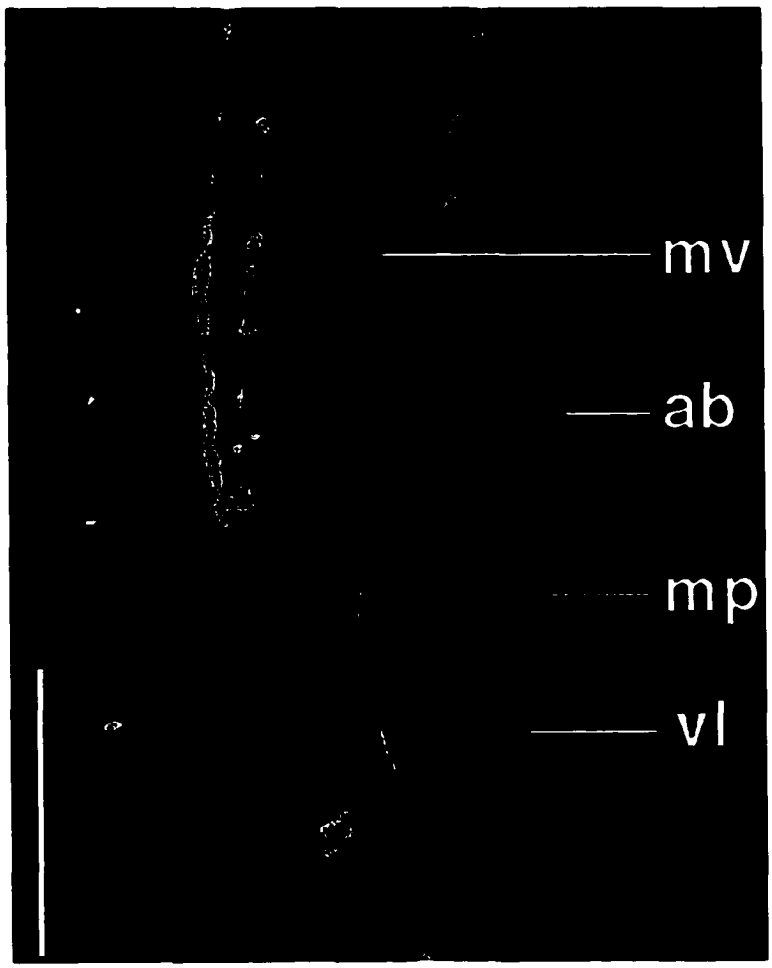



Figure 7.3. Ventral view of middle trunk region. ab – anteriorly-directed branch of musculi paralaterales, mp – musculi paralaterales, mv – musculi ventrales, vl – musculi ventrolaterales. Scale bar is 15 μm.

Segmentally-arranged dorsoventral muscles existed in two positions (lateral and medial) relative to the longitudinal muscles and gut tube (Figs. 7.2, 7.5). Precise locations of muscle fiber insertion on the dorsal and ventral cuticle were not discerned (but see Ruppert, 1975, pp. 167) The lateral dorsoventral muscles began at approximately U25 and extended posteriorly to the top of the caudal furca (U80). Most of the lateral dorsoventral fibers were positioned external to the musculi laterales, but this was not always possible to determine in whole mount preparations. The dorsoventral muscles inserted on the dorsal cuticle, curved ventrally around the musculi laterales, and inserted on the ventral cuticle. The precise number of dorsoventral muscles was difficult to visualize, but one specimen had approximately 30 pairs of dorsoventral fibers. All dorsoventral muscles were evenly spaced in the trunk region. Some muscles appeared to bifurcate ventrally before inserting on the cuticle.

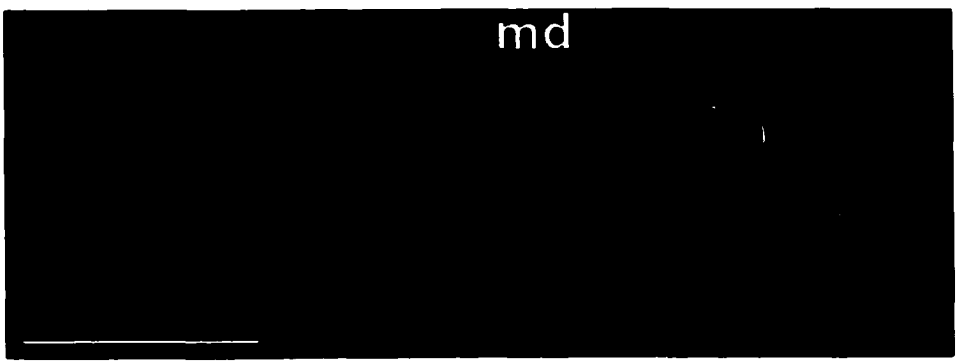



Figure 7.4. Lateral view of *Draculiciteria tessalata* showing musculi dorsales (md). Scale bar is 70 μm.

The medial dorsoventral muscles began posterior to the pharyngeal-intestinal junction (U30) and appeared to line both sides of the intestine. They inserted on the dorsal cuticle, curved ventrally around the intestine, encompassing the musculi dorsales, musculi ventrolaterales and musculi ventrales, and inserted on the ventral cuticle. The medial dorsoventral muscles appeared to correspond in a relative 1:1 ratio to the lateral dorsoventral muscles. The medial dorsoventral muscles were found in each branch of the caudal furca.

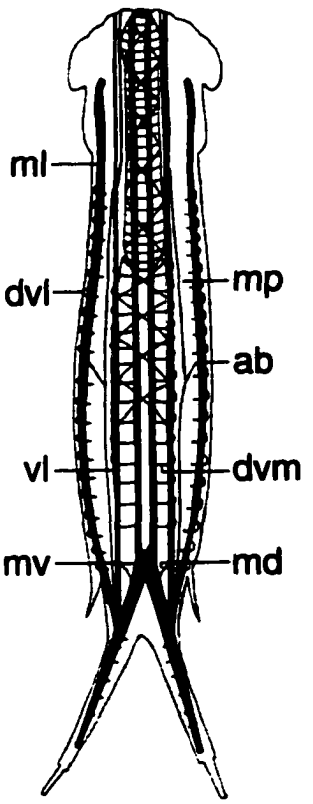



Figure 7.5. Schematic of *Draculiciteria tessalata* revealing the major muscles in ventral view. ab – anteriorly-directed branch of musculi paralaterales, dvm - medial dorsoventral muscles, dvl – lateral dorsoventral muscles, md – ventral connection of musculi dorsales, ml – musculi laterales, mp – musculi paralaterales, mv – musculi ventrales, vl – musculi ventrolaterales

## Discussion

The muscles of *Draculiciteria tessalata* described in this study correspond in relative position and orientation to those discussed by Ruppert (1975). In his monograph, Ruppert described circular muscles on the pharynx, dorsoventral muscles in the trunk region, and four pairs of longitudinal muscles spanning the length of *D. tessalata*. Our phalloidin staining revealed an additional pair of longitudinal muscles in the lateral body region, and helicoidal muscles spiraling around both pharynx and intestine. We did not observe any circular muscle fibers in the caudal region as alluded to by Ruppert (1975).

The musculature of *D. tessalata* is unusual in that it possess segmentally-arranged dorsoventral muscles in place of complete circular bands in the trunk. As noted by Ruppert (1975), most chaetonotidan gastrotrichs possess circular muscles only on the pharynx and rarely in the trunk. However, two species of *Xenotrichula* have been found to possess circular bands anteriorly on the intestine and somatic circular muscles in the lateral trunk region (Ruppert 1975; Travis 1983). Interestingly, *X. intermedia* possesses both circular muscles and dorsoventral muscles. This latter condition would seem to be a plausible evolutionary intermediate condition between species of *Xenotrichula* with complete circular muscles and *D. tessalata* with only dorsoventral bands.

If, as suggested by Ruppert (1975), the dorsoventral muscles are evolutionary derivatives of complete circular bands, then an analysis of their ultrastructure should reveal how these cellular modifications evolved. As yet, there are no comprehensive details on the ultrastructure of circular muscles in Chaetonotida (but see Ruppert, 1991 for *D. tessalata*), but data on some macrodasyidans may reveal how these evolutionary modifications may have occurred.

We thus propose the following evolutionary scenario. Somatic circular muscles of the trunk region in macrodasyidans are presumably formed via lateral branches of splanchnic circular muscles (Fig. 7.6A; see Teuchert & Lappe, 1980). A split between somatic and splanchnic bands may have freed up the somatic component to take on a dorsoventral orientation as in some species of *Xenotrichula* (Fig. 7.6B). Splitting of the splanchnic component combined with a middorsal and -ventral insertion into the cuticle of the splanchnic muscles would result in the dorsoventral orientation we observed in *D. tessalata* (Fig. 7.6C ). Detailed ultrastructural examinations of *X. intermedia* and other members of the Xenotrichulidae may further confirm this hypothesis.

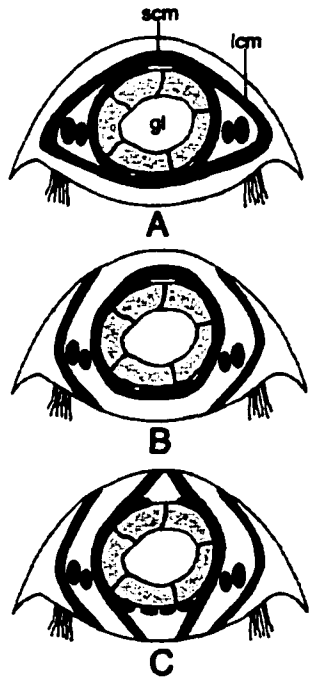



Figure 7.6. Hypothetical evolutionary sequence of changes in the orientation of circular muscles seen in cross section. (A) Condition present in many macrodasyidans. (B) Intermediate condition as seen in some members of *Xenotrichula*. (C) Derived condition present *in Draculiciteria tessalata*. Dark circles represent longitudinal muscles. gl – gut lumen, lcm – lateral branches of circular muscles forming somatic components, scm – splanchnic circular musculature.

The adaptive nature of dorsoventrally-oriented bands versus circular bands should also be sought. Observations on macrodasyidan species with complete circular muscles (splanchnic and lateral somatic components), suggest that these muscles function in antagonizing longitudinal muscles and radial dilations of the gut tube (Travis ,1983; Hochberg & Litvaitis 2001d). It is uncertain how dorsoventral fibers might directly antagonize gut dilations or contracted longitudinal muscles, but presumably contractions in the dorsoventral plane would flatten and pressurize the body leading to a similar effect. The significance of specific insertion sites for the dorsoventral muscles (ventral hydrofoil scales and dorsal paramedially positioned scales; Ruppert ,1975, p. 167) remains to be determined.

# CHAPTER 8

# THE MUSCULATURE OF *NEODASYS AUSTRALIENSIS* (GASTROTRICHA, CHAETONOTIDA)

## Introduction

The gastrotrich suborder Multitubulatina is comprised of a single monogeneric family, Neodasyidae, that includes only three species: *Neodasys chaetonotoideus* Remane, 1936, *N. uchidai* Remane, 1961, and *N. cirritus* Evans, 1991. Since its first discovery, *Neodasys* has held a curious taxonomic and phylogenetic position within the Gastrotricha.

The peculiarity of *Neodasys* was recognized early on by Remane (1927) who originally classified the genus as part of the order Macrodasyida based on body form and the presence of lateral and caudal adhesive tubes. The genus was later moved to the order Chaetonotida based on the Y-shaped orientation of the pharyngeal lumen, an ordinal-level character (Remane 1936). Since Remane, several studies have contributed important ultrastructural information confirming the taxonomic placement of *Neodasys* within the Chaetonotida: pharyngeal innervation is similar in species of Paucitubulatina and *Neodasys* (Ruppert 1982), and species from both suborders have reduced musculature in the trunk region (Teuchert & Lappert 1980; Travis 1983).

Despite early confusion over its taxonomic placement, the phylogenetic position of *Neodasys* within the Chaetonotida is firmly established using morphological criteria.

Remane (1961) considered the genus to represent the most primitive chaetonotidan line, later confirmed by Ruppert (1982) using ultrastructural aspects of the pharynx and Travis (1982) using characters of the body wall. A cladistic analysis of the phylum also placed *Neodasys* at the base of the Chaetonotida (Hochberg & Litvaitis 2000c). However, species of *Neodasys* possess several aberrant characters that also argue for its placement as the *most primitive* gastrotrich (i.e., the ancestor of both Macrodasyida and Chaetonotida), and not just a basal clade within the Chaetonotida.

Among the more unusual characters include the plesiomorphic structure of the adhesive organs, nervous system and muscular system. Tyler et al. (1980) found the lateral and caudal adhesive organs to be different from both macrodasyidans and other chaetonotidans. The adhesive organs were not of the duo-gland type (adhesive and releaser gland); instead, the organs consisted only of an adhesive gland and a ciliated sensory cell, the latter cell type being a hypothetical evolutionary precursor to the releaser gland present in Chaetonotida Paucitubulatina and Macrodasyida. The structure of the nervous system also suggests a primitive condition. Ruppert (1982) found a species with six lateral nerve cords (3 per side) that coalesced into two cords posteriorly (Ruppert 1982); multiple nerve cords are hypothesized to be the plesiomorphic condition in Gastrotricha (Travis 1983). The ultrastructure of the muscular system is also peculiar. The muscles are cross-striated but possess differently organized Z material and lack a T-system characteristic of cross-striated muscles in other gastrotrichs (Ruppert 1991).

By all accounts, species of *Neodasys* possess several aberrant characteristics that are best explained in a historical context (i.e., the possession of multiple plesiomorphic characters are not obviously functionally correlated). The aim of the current study is to

examine the muscular topology of a new species of *Neodasys* and see if it is consistent with what we know about the phylogenetic position of the genus.

## Materials and Methods

Specimens of a new species of *Neodasys* (Fig. 8.1) were collected from Cylinder beach, North Stradbroke Island, Queensland, Australia. A complete description of *Neodasys australiensis* n. sp. will appear in a separate publication (type material is deposited at the Queensland Museum, Australia). Animals were common in the medium grain sand at the low tide level. Gastrotrichs were extracted from the sediment using an anesthetization/decantation technique with 7.5% magnesium chloride (Pfannkuche & Thiel 1988).

For whole-mount muscle staining, animals were relaxed for 15 minutes in 7% $MgCl_2$ solution prior to fixation in 5% formaldehyde in 0.1M PBS (1 hr). Animals were rinsed in PBS, permeabilized for 1 hr in 0.2% Triton X-100 in PBS, stained 50 min with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR) and rinsed in PBS before mounting with Gel/Mount. Specimens were viewed on an Olympus BH2 microscope equipped with SPOT digital camera at the Centre for Microscopy and Microanalysis, University of Queensland. Measurements of gastrotrichs were performed with an ocular micrometer and the positions of organs are expressed in reference to percentage body units (total body length being 100 units (U)).

Figure 8.1. *Neodasys australiensis* n. sp. Scale bar = 100 μm

## Results

Eighteen specimens of *Neodasys australiensis* n. sp. were analyzed as whole mounts. Muscles were present in four orientations: circular, semicircular, helicoidal and longitudinal. All muscles were cross striated.

### Circular Muscles

Splanchnic circular muscles were present as the innermost muscle layer on the pharynx. All muscles were thin and compact. At the pharngeointestinal junction, the muscles appeared slightly more spread apart but remained extremely thin, almost filament-like strands (ca. 1 μm; Fig. 8.2). These muscles also appeared to be the innermost muscular layer. Splanchnic circular muscles were absent from the midgut region, U30-U60. Circular muscles resumed around U60 as both splanchnic and somatic components. In this region, circular muscles were spread farther apart than on the anterior third of the intestine (Fig. 8.3). Somatic circular muscles were also present as discreet rings in the caudal region and surrounded the ventrolateral longitudinal muscles. The somatic circular muscles were distinctly thicker (2-3 μm) than the splanchnic musculature at the anterior end. There appeared to be a 'doubling up' of the circular muscles around the male caudal organ, U75-U85 (Fig. 8.4). In this region, the circular muscles were more closely packed than those immediately anterior to them.

### Semicircular Muscle

A single semicircular muscle was present dorsally around U60 (Fig. 8.3, 8.6). The muscle arched dorsally over the intestine and the paired dorsal longitudinal muscles. Left and right insertions were on the ventrolateral muscles.

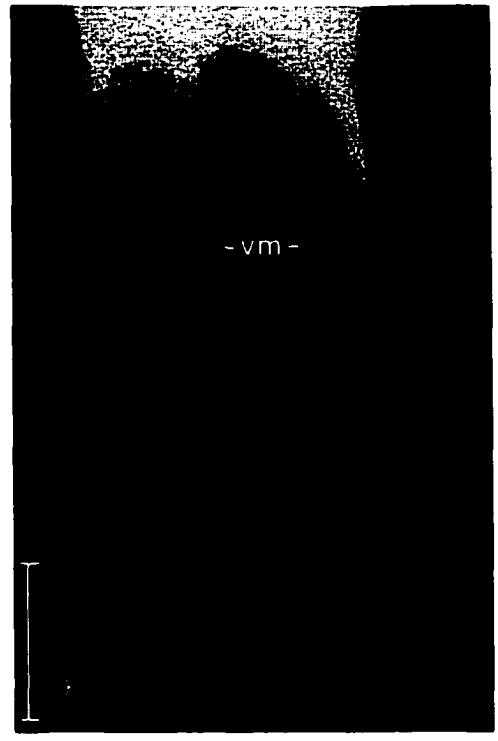



Figure 8.2. Ventral view of phalloidin-stained specimen showing region posterior to the pharyngeointestinal junction (pij). cm, splanchnic circular muscles; vm, ventral longitudinal muscles. Scale bar = 20 μm.

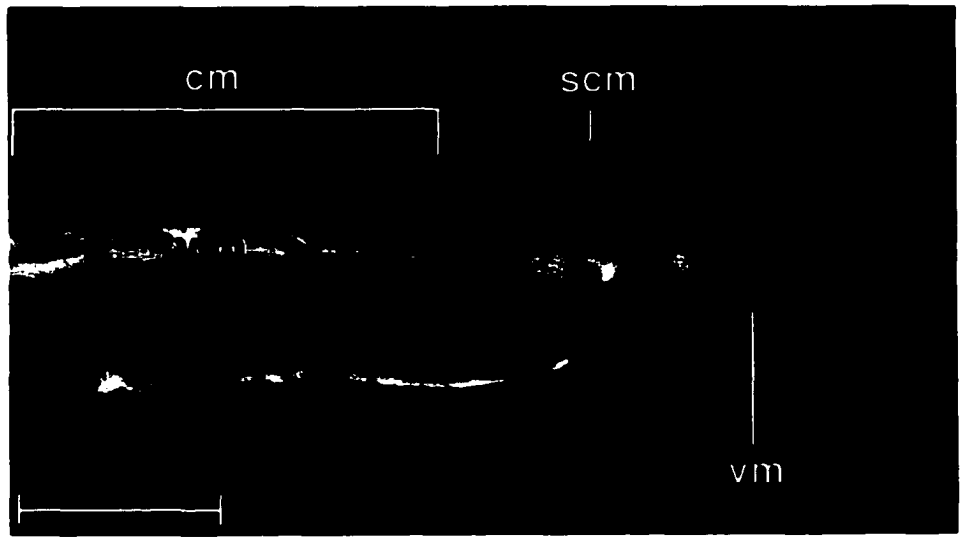



Figure 8.3. Ventrolateral view of phalloidin-stained specimen showing posterior trunk region. Right is anterior. Cm, somatic circular muscles; scm, semicircular muscle; vm, ventral longitudinal muscle; vlm, ventrolateral longitudinal muscle. Scale bar = 50 μm.

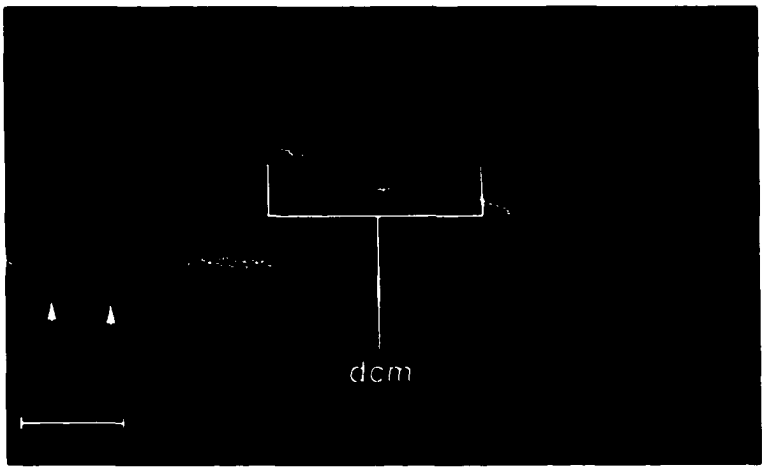



Figure 8.4. Phalloidin-stained wholemount showing posterior trunk region around male caudal organ. dcm, doubled-up circular muscles. Arrows point to unknown region with transverse striations. Scale bar = 20 μm

Helicoidal Muscles

Helicoidal muscles were only observed on the pharynx (Fig. 8.6). These muscles were extremely thin (ca. 1 μm) and difficult to visualize. Approximately 8 helical crosses were observed on the dorsal side of the pharynx in one specimen. The helices made 40°-45° crosses relative to the longitudinal axis.

Longitudinal Muscles

Four pairs of longitudinal muscles were present and extended the length of the body in dorsal, lateral, ventrolateral, and ventral positions. Dorsally, a single pair of thin (1-2 μm) longitudinal muscles inserted on the lateral side of the pharynx close to the mouth. The muscles curved medially over the posterior aspect of the pharynx and remained in a dorsomedial position until ca. U70 where they took up a dorsolateral

position over the intestine. The posterior insertion was not determined. A single thin pair of lateral longitudinal muscles inserted anteriorly on the body wall close to the mouth. Each muscle produced two thin (ca. 1 μm) lateral branches at approximately U3 and U4 that inserted on the lateral body wall of the head. The lateral longitudinal muscles remained in close contact with the digestive tract and the ventrolateral muscles for its entire length. The bilateral ventrolateral bands were the thickest longitudinal muscles (4-6 μm) in the body and consisted of several individual muscle cells in parallel. In the region of the pharynx, the ventrolateral bands appeared to consist of but a single muscle fiber (cell?). The muscle paralleled the pharynx for approximately half its length before bowing laterally and then crossing ventral to the lateral longitudinal muscle, inserting close to the mouth margin. In the trunk region, the muscle bands remained close to the intestine and outside the splanchnic circular muscles. Their posterior insertion was in the bilobed caudum. A single pair of ventral muscles inserted anteriorly at the mouth margin and extended the length of the body inserting posteriorly in the midline of the body behind the anus.

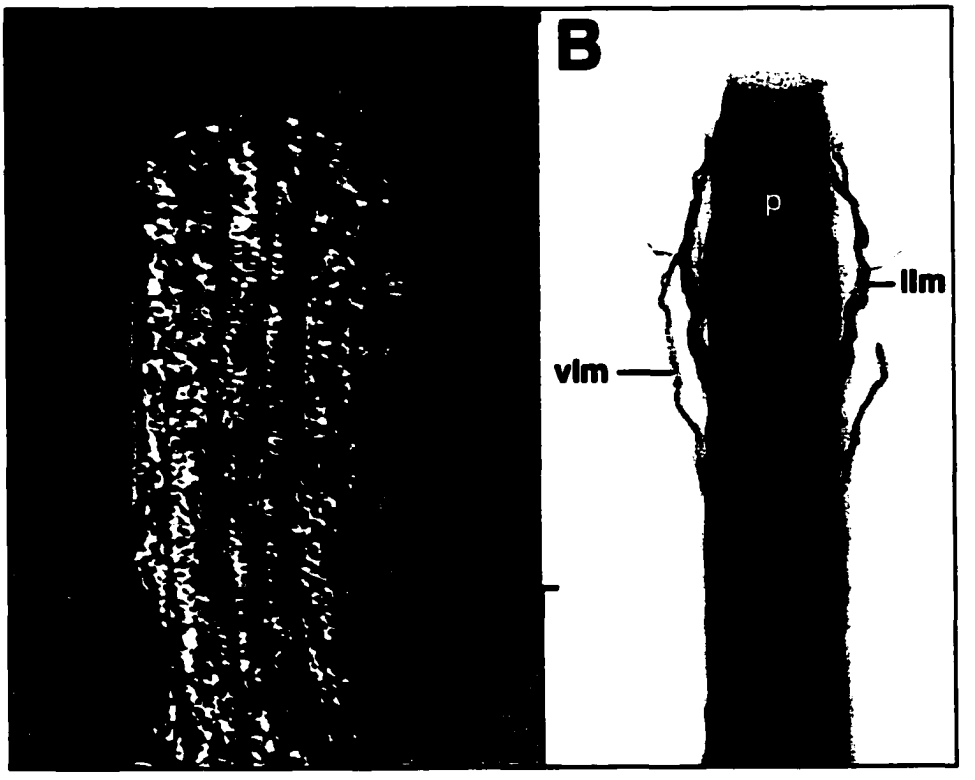



Figure 8.5. *Neodasys australiensis* n. sp. A) Light micrograph of anterior end. B) Phalloidin-stained wholemount showing anterior end. llm, lateral longitudinal muscle; vlm, ventrolateral longitudinal muscle. Scale bar = 20 μm.

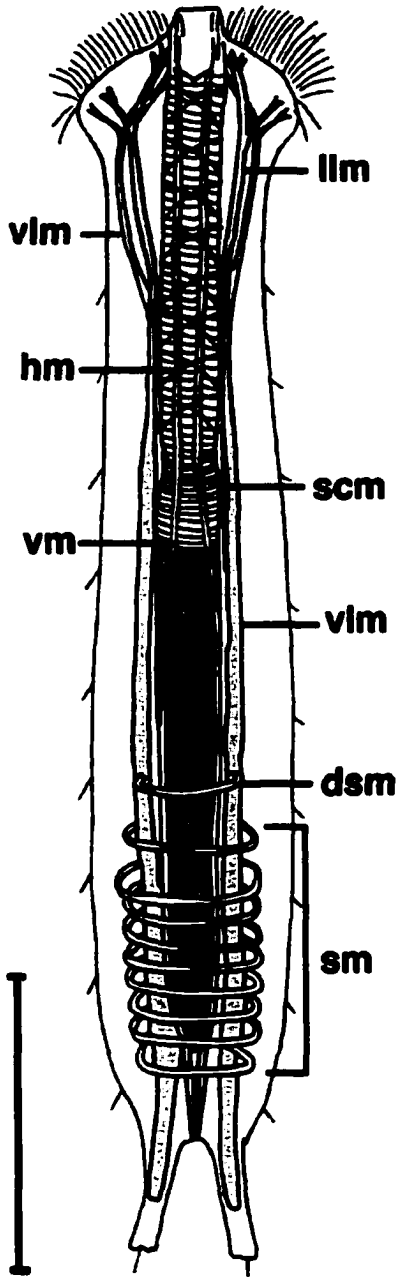



Figure 8.6. Schematic diagram of muscle topology in Neodasys australiensis n. sp. in ventral view. The dorsal semiciruclar muscle (dsm) is illustrated to show its position. hm, helicoidal muscles; llm, lateral longitudinal muscle; sm, somatic circular muscles; scm, splanchnic circular muscles; vm, ventral longitudinal muscle; vlm, ventrolateral longitudinal muscle.

## Discussion

### Muscle Topology

The body musculature of species of *Neodasys australiensis* n. sp. shows a greatly reduced development similar to other species in the genus (Ruppert & Travis 1982; Travis 1983). In agreement with previous observations, circular muscles are poorly developed in the trunk region, longitudinal muscles are poorly developed throughout (i.e., thin diameter), and the principal longitudinal muscles are the ventrolateral bands. In contrast to previous findings on other species, *N. australiensis* possesses splanchnic circular muscles on the intestine and somatic circular muscles in the caudal region. Another novel find is the presence of helicoidal muscles on the pharynx (*sensu* Hochberg & Litvaitis 2001a).

In a topological context, the musculature of *Neodasys* agrees with that of most other gastrotrichs. Circular muscles are the innermost layer of the pharynx, and the longitudinal muscles are present in the same four orientations as that of all other species investigated (Hochberg & Litvaitis 2001a,b,d,e,f). However, it is noteworthy that these muscles are extremely thin considering the size of the animal. The same is true for the splanchnic circular muscles on the anterior region of the intestine and helicoidal muscles on the pharynx. In the case of the splanchnic circular muscles, they appeared more as web-like filaments than they did as true circular fibers. In macrodasyidan gastrotrichs of similar size, all sets of circular and longitudinal bands are generally much wider (2-4 μm, Hochberg & Litvaitis 2001d); this is also true of many smaller chaetonotidan gastrotrichs (e.g., *Xenotrichula intermedia*; Hochberg & Litvaitis 2002).

The relative position of helicoidal muscles on the pharynx remains undetermined for this species. In most other species, the helicoidal muscles lie external to the circular and longitudinal muscles of the pharynx. In *N. australiensis*, this is difficult to determine. The same problem occurred with the splanchnic circular muscles on the anterior intestine. Splanchnic circular muscles are generally external to longitudinal bands on the intestine in all species of Gastrotricha (see Ruppert 1991). However, this does not appear to be true of *N. australiensis*. The use of TEM or confocal laser scanning microscopy is necessary to resolve this dilemma.

Functional Considerations

*Neodasys australiensis*, like other species in the genus, is a relatively slow moving gastrotrich, but can rapidly withdraw its body to nearly one-third original size when provoked (see also Ruppert & Travis 1982). However, re-extension of the body is comparatively slower, likely due to the general lack of somatic circular muscles in the trunk region. The probably function of the somatic circular muscles is to antagonize the large ventrolateral bands that are the main source of directional change and body withdrawal in both macrodasyidan and chaetonotidan gastrotrichs (Hochberg & Litvaitis 2001b, d). The absence of somatic circular rings from the anterior half of the intestine is difficult to account for in a functional context, considering the only other species of similar size without somatic circular muscles are the thaumastodermatids. In these macrodasyidans, the thick sculptured cuticle is hypothesized to oppose the longitudinal contraction of the body and thereby restore body shape when the ventrolateral longitudinal muscles are relaxed; the muscle-derived Y-cells of thaumastodermatids may also antagonize longitudinal contractions (Travis 1983). It is probable that the loss of

somatic circular muscles is correlated with the evolution of the sculptured cuticle in these species (Travis 1983). Why *N. australiensis* should lack somatic circular muscles remains unknown.

Functional reasons for the comparative lack of development of the longitudinal muscles are also difficult to explain. Their weak development does not account for the slow forward movement of the species since this can only be attributed to the ventral ciliation. Other anomalies without functional causation include the presence of a dorsal semicircular muscle in the trunk region and the absence of helicoidal muscles from the intestine. Some macrodasyidan species also lack helicoidal muscles on the intestine, e.g., species of *Dolichodasys*, *Paradasys*, *Macrodasys*, *Paraturbanella* and *Turbanella* (personal observations). Hochberg and Litvaitis (2001a) hypothesized that helicoidal muscles might fulfill several functions: pressurize the pharynx and intestine during feeding by antagonizing radial dilations of the gut tube; move food items down the digestive tract through peristaltic contraction; prevent kinking of the intestine during severe body contraction or bending; and stiffen the hydrostatic gut into a notochord-like structure, thereby straightening the body. In Chaetonotida, the helicoidal muscles may also contribute to closure of the pharyngeal-intestinal valve by creating a backpressure from the intestine. Interestingly, helicoidal muscles appear to be absent only from the intestine of highly elongate species (> 500 μm) with smooth cuticles, suggesting a negative correlation between the presence of intestinal helicoidal bands and long body length. Regardless, accounting for the absence of helicoidal muscles is a mystery, provided hypotheses on their presence appear grounded.

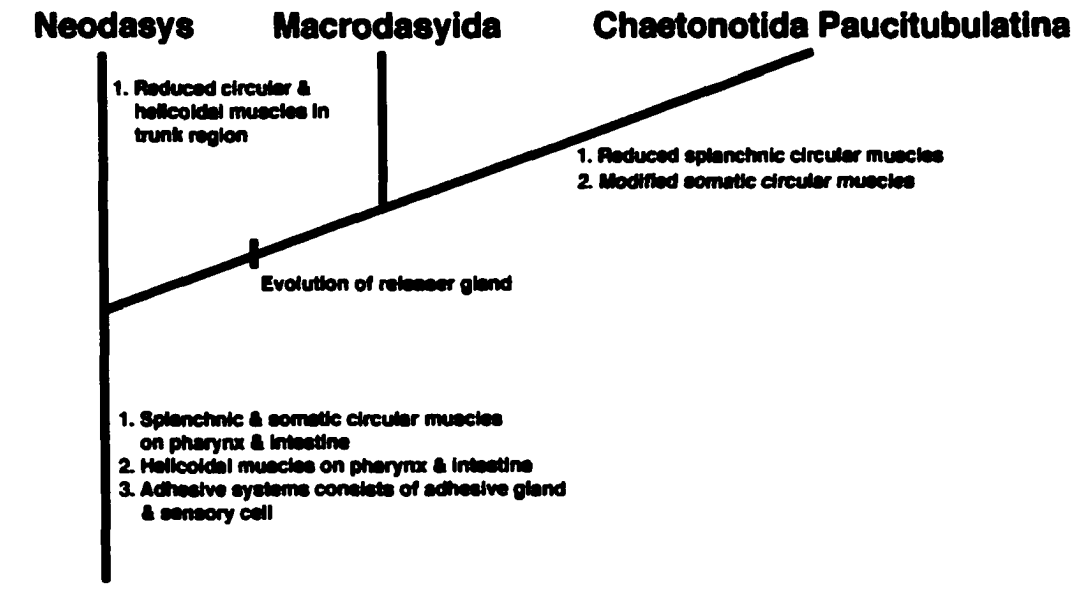
Phylogenetic Implications

A close relationship between *Neodasys* and species of Chaetonotida Paucitubulatina, and between *Neodasys* and species of Macrodasyida, is evident based on morphology. Cladistic analyses have consistently placed *Neodasys* at the base of the chaetonotidan lineage while understanding that the genus also has close ties to macrodasyidan gastrotrichs (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000c). Evidence derived from the muscular system suggests a similar phylogenetic position for the genus. The weakly developed somatic and splanchnic circular muscles in the trunk region is analogous to the condition in more derived chaetonotidans. Is this condition a result of phyletic heritage or convergence? The most parsimonious explanation would suggest that the condition in both suborders is homologous. However, it is also clear that the exact location of reduction is different in both taxa, suggesting otherwise. For example, in *Neodasys*, helicoidal muscles are entirely absent from the trunk region while somatic circular muscles are confined to the posterior trunk. In *Xenotrichula*, both helicoidal muscles and somatic circular muscles are present in the trunk region, and while most species of Paucitubulatina lack somatic circular muscles, all possess a full complement of helicoidal muscles along the digestive tract.

While it remains difficult to explain the muscular condition in *Neodasys*, a probable explanation for the poor development of certain muscle groups in Chaetonotida Paucitubulatina is related to their comparatively smaller size and presence of a rigid sculptured cuticle. However, not all chaetonotidans have poorly developed trunk muscles despite their size and cuticular armament. *Xenotrichula intermedia* has well-developed somatic circular muscles and longitudinal muscles and is also placed in the most

plesiomorphic clade of Paucitubulatina (Hochberg & Litvaitis 2002). In many respects, the musculature of *Xenotrichula* appears closer to that of Macrodasyida than does that of *Neodasys*.

Based on other morphological criteria (e.g., body shape, distribution of adhesive glands), *Neodasys* does initially appear to be a good intermediate form between Macrodasyida and Chaetonotida Paucitubulatina (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000c). However, this is not evident from an examination of the muscular system alone. Perhaps then the most parsimonious placement of *Neodasys* as the sister group to the Paucitubulatina is not the correct one. Could *Neodasys* represent the most "primitive" genus of Gastrotricha? Evidence from the ultrastructure of the adhesive organs would seem to suggest that the genus has retained the plesiomorphic structure of the organs while the releaser glands developed *de novo* in the suborder Paucitubulatina and the order Macrodasyida (Tyler et al. 1980). If this is the case, it may argue for a basal position for the genus, and the releaser gland would then be a synapomorphy uniting Macrodasyida and Paucitubulatina (see Fig. 8.7), or, as Tyler et al. suggests (1980), the releaser gland is convergent between the taxa. Alternatively, the releaser gland may have been secondarily lost in *Neodasys* (Fig. 8.7).



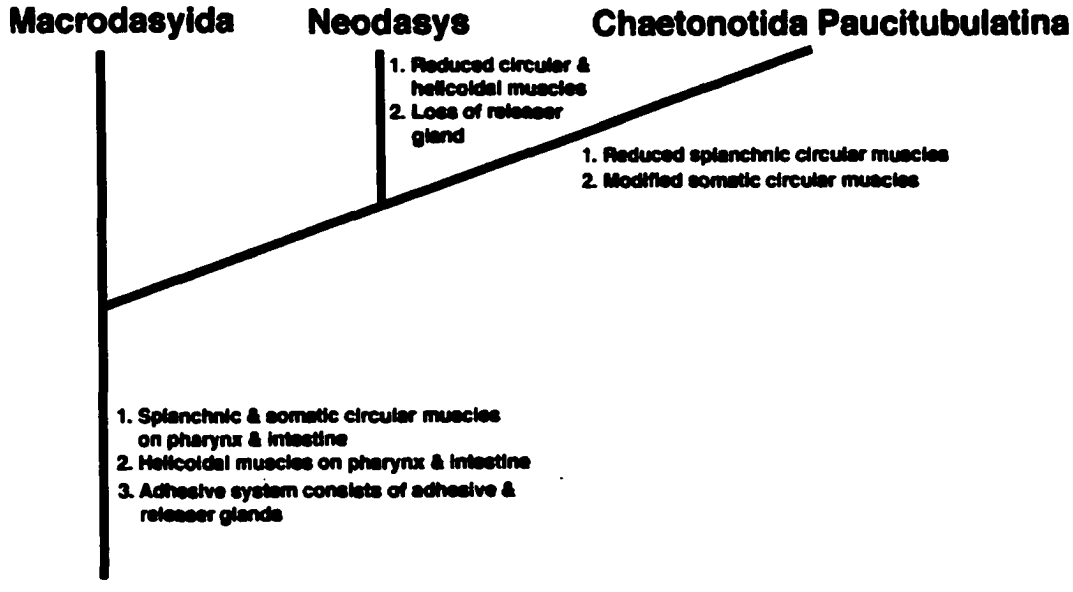




Figure 8.7. Two cladograms with different positions for *Neodasys* based on the evolution of the adhesive system. Muscle topologies are mapped onto both cladograms for comparison. The top cladogram unites Macrodasyida and Paucibulatina with the synapomorphy, releaser glands. The bottom cladogram (traditional position of *Neodasys*) assumes secondary loss of the releaser glands.

# CHAPTER 9

# EVOLUTION OF THE GASTROTRICH MUSCULAR SYSTEM

## Introduction

Gastrotrichs remain one of the least understood groups of aquatic invertebrate animals. Details of their development and homology of characters remain unclear. Furthermore, the relationship of Gastrotricha to other extant metazoans is uncertain; results from recent molecular (Carranza et al., 1997; Littlewood et al. 1998; Zrzavy et al., 1998) and morphological studies (Wallace et al. 1996; Zrzavy et al., 1998) are in conflict. To arrive at a better understanding of gastrotrich systematics and evolution, cladistic analyses and detailed studies of the muscular system were performed. The purpose of this chapter is to summarize the results of earlier work, estimate the muscular ground pattern for the phylum, and present hypotheses on the evolution of the gastrotrich muscular system and the origin of the Gastrotricha.

## Summary of Results

### Cladistic Analysis

Characters derived from the body wall, digestive tract, muscular system, nervous system and reproductive system were used to construct hypotheses on the phylogeny of the Gastrotricha. Results suggest that the Gastrotricha and its inclusive orders, Chaetonotida and Macrodasyida, are monophyletic and defined by the structure of the pharynx and the adhesive organs among other characters. Within the Macrodasyida, four

of six families are shown to be monophyletic: Dactylopodolidae, Macrodasyidae, Thaumastodermatidae and Turbanellidae. Dactylopodolidae was further confirmed as the most basal family within the order based on the retention of several plesiomorphies. The other three families have well-defined autapomorphies but will require further investigation to increase inter- and intrafamilial phylogenetic resolution. Within the Chaetonotida, five of seven families are monophyletic: Dasydytidae, Neodasyidae, Neogosseidae, Proichthydidae, and Xenotrichulidae. The largest and structurally most diverse family, the Chaetonotidae, appears to be polyphyletic. The Neodasyidae is the most basal family within the order, and the Xenotrichulidae is the most basal family within the suborder Paucitubulatina.

Muscular System

The fluorescent F-actin stain, Alexa Fluor 488 phalloidin, was applied to whole mounts of 24 species of Gastrotricha from both orders to characterize the musculature. Muscle patterns were mapped, their functions inferred, and the direction of evolution hypothesized for several families. The musculature of all gastrotrichs is arranged as a series of circular, helicoidal, and longitudinal bands around the digestive tract. Circular muscles are present as discreet rings in somatic and splanchnic positions but may be absent from the intestine or body wall of several derived species including members of Thaumastodermatidae, Chaetonotidae and Xenotrichulidae. Helicoidal muscles are the thinnest-diameter muscles in the gastrotrich body and generally surround both splanchnic circular muscles and longitudinal bands. Longitudinal muscles are arranged radially around the digestive tract in dorsal, lateral, ventrolateral and ventral positions. Some species have extra sets of longitudinal muscles (e.g., species of Chaetonotida).

Order Macrodasyida. In macrodasyidan gastrotrichs, the musculature of species of *Dactylopodola* (Dactylopodolidae) is considered to be closest to the ground pattern of the order and consists of the following from gut to body wall: splanchnic circular muscles on the pharynx and intestine, longitudinal muscles in dorsal, lateral, and ventral positions, helicoidal muscles, ventrolateral longitudinal muscles, and somatic circular muscles. Longitudinal muscles are largely concentrated on the ventral and ventrolateral sides of the body where they aid in body flexion, including directional changes during ciliary swimming, body torsion, and escape responses. Helicoidal muscles are hypothesized to counteract dilations of the pharynx and intestine during feeding. Extraordinary muscle orientations with undetermined functions include a pair of cross-over muscles in the caudal region (in species of Dactylopdolidae, Turbanellidae, and Thaumastodermatidae) and semicircular muscles on the ventral aspect of the phaymx (in species of Dactylopodolidae and Turbanellidae). Species of the Turbanellidae and Lepidodasyidae lack helicoidal muscles on the intestine, and thaumastodermatids lack somatic circular muscles.

Order Chaetonotida. In the Chaetonotida, the musculature of species of *Neodasys* is considered closest to the ground pattern of the order. Splanchnic circular muscles line the pharynx and part of the intestine and somatic circular muscles are present caudally. Helicoidal muscles are present only on the pharynx. Longitudinal muscles are concentrated ventrally and ventrolaterally. Reductions in splanchnic and somatic circular muscles are hypothesized to be convergent between *Neodasys* and more derived chaetonotids. The musculature of *Xenotrichula intermedia* (Xenotrichulidae) is considered to be closest to the ground-pattern of the Paucitubulatina and consists of the

following: complete splanchnic circular muscles on the pharynx and part of the intestine, incomplete splanchnic circular muscles on the intestine, helicoidal muscles, four to five pairs of longitudinal muscles that extend the length of the body, a specialized pair of dorsal longitudinal muscles (Rückenhautmuskel), diagonal muscles, incomplete somatic circular muscles, and dorsoventral muscles. The Rückenhautmuskel is a special branch of the musculi dorsales and functions to maintain the position of developing eggs. The general organization of muscles in the Chaetonotidae is similar to *X. intermedia* with the following modifications: three to four pair of longitudinal muscles, a branched Rückenhautmuskel, no circular muscles in the trunk region, and a single pair of dorsoventral muscles in the caudal region.

Muscular Ground Pattern of the Gastrotricha

Reconstruction of the ancestral topological organization of the muscular system in Gastrotricha is based on muscle patterns from five species representing the most basal clades: Macrodasyida, *Dactylopodola baltica* Remane, 1927, *D. agadasys* Hochberg 2002, and *D. australiensis* Hochberg 2002; and Chaetonotida, *Neodasys australiensis* Hochberg, 2002, *Xenotrichula intermedia* Remane, 1934.

The ancestral topological organization of the muscular system consists of muscles in the three primary orientations: circular, helicoidal, and longitudinal. Splanchnic circular muscles are present inside longitudinal bands on the pharynx but outside longitudinal bands on the intestine. Helicoidal muscles lie outside both sets of muscles along the entire digestive tract. Somatic circular muscles enclose only the ventrolateral longitudinal muscles in the trunk region.

Insertion points for the longitudinal muscles remain only partially resolved. Anterior insertions for the dorsal and ventral longitudinal muscles are on the pharynx close to the mouth. The anterior insertion for the ventrolateral bands is correlated with the presence of anterior adhesive tubes in macrodasyidans (see Chapter 3); similar structures are absent from all chaetonotidans including *Neodasys*. Therefore, the plesiomorphic site of ventrolateral muscle insertion remains unknown, but is undoubtedly in the middle to anterior region of the pharynx (Fig 9.1).

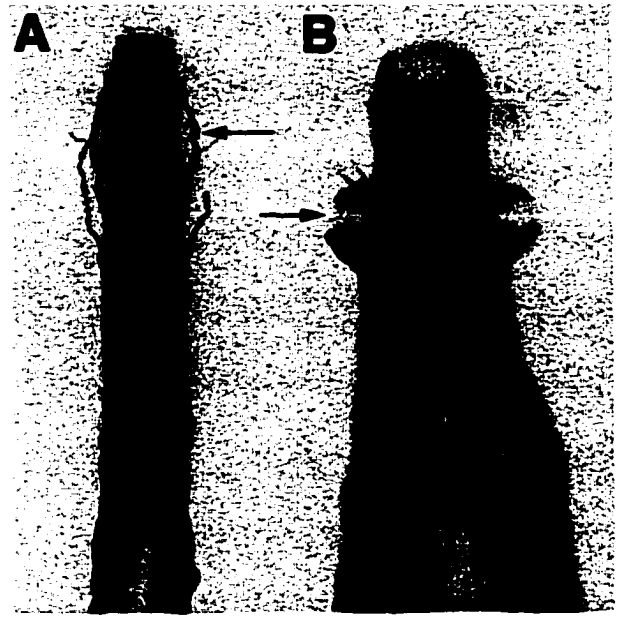



Figure 9.1. Phalloidin stained whole mounts of A) *Neodasys australiensis* and B) *Dactylopodola australiensis* showing anterior end of ventrolateral muscles (arrows). Digitial photographs were "inverted" to enhance contrast.

The caudal insertion sites for the longitudinal muscles are as follows: dorsal longitudinal muscles insert at body midline in caudal region; lateral longitudinal muscles join ventrolateral bands to enter each caudal ramus; dorsal longitudinal muscles insert

close to body midline in caudal region. The presence of a bilobed caudal region in both chaetonotidans (called a caudal furca) and macrodasyidans indicates that the ancestral gastrotrich also had a forked caudum with similar posterior muscle insertions (e.g. Fig 9.2).

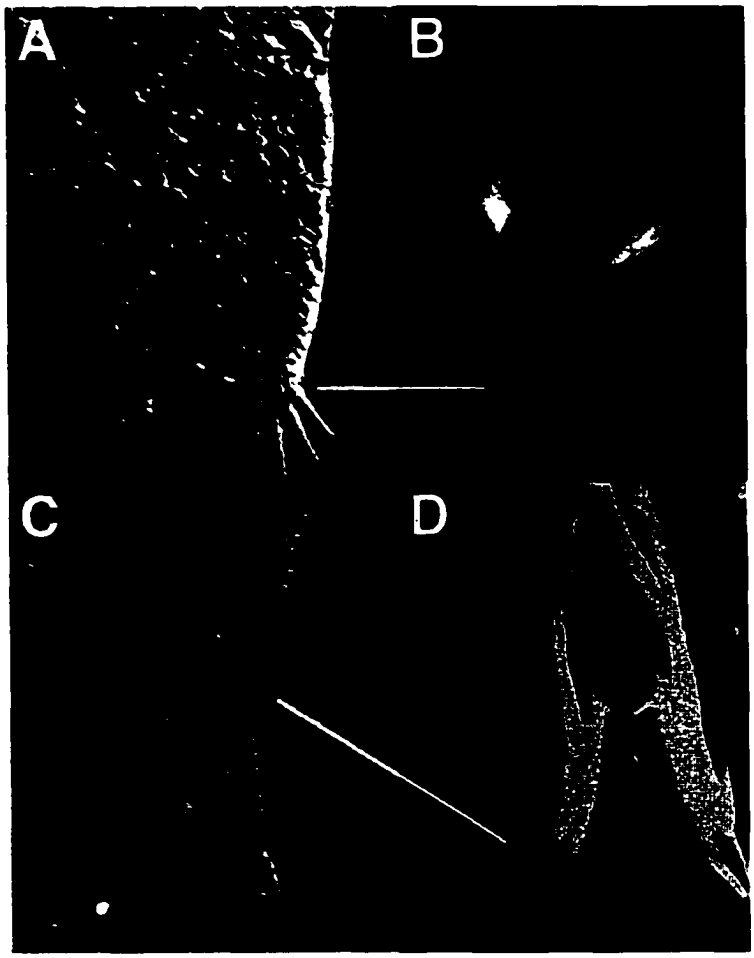



Figure 9.2. Posterior region of two gastrotrichs. A) Light micrograph of *D. agadasys* and B) phalloidin-stained whole mount. C) Light micrograph of *X. intermedia* and D) phalloidin-stained whole mount. Lines point to region where ventrolateral muscles insert in caudum.

## Origin of the Gastrotricha and Evolution of the Gastrotrich Muscular System

The origin of gastrotrichs and the identification of their living sister group remains today as perplexing as it was since speculations last century by Hyman (1951), Steinbock (1958, 1963), Remane (1958) and Beklemischev (1969). The overwhelming view of earlier researchers is that gastrotrichs were derived from some turbellarian stock, perhaps close to the Acoela, and Nematoda was considered the most probable sister taxon based predominantly on the structure of the myoepithelial pharynx. Since then, molecular characters have shown several several groups to be sister taxa to the Gastrotricha including Acanthocephala (Carranza et al. 1997), Gnathostomulida (Littlewood et al. 1998), Nematomorpha (Carranza *et al.*, 1997), and Platyhelminthes (Winnepenninckx et al. 1995). Curiously, a sister group relationship between gastrotrichs and nematodes has never been substantiated using molecular characters. Which if any of these groups represents the closest sister taxon to the Gastrotricha remains a lively source of speculation.

Do details of the muscular system provide clues to the origin and relationships of gastrotrichs? Theories on the origin of the Bilateria are central to answering this question. Numerous hypotheses exist on the original bilaterian stem species and its characteristics, e.g., size (microscopic vs macroscopic), body cavity (acoelomate, pseudocoelomate, coelomate), segmentation, life history (direct vs indirect developer), and body organization (solitary vs colonial) (see review by Rieger & Ladurner 2001). Because gastrotrichs have been envisioned as "primitive" due to their minute size, lack of coelom and relatively simple organization, they are often placed close to the original bilaterian. Much of the reason for this is due to the prevailing view in older literature (e.g., Hyman

1951) that flatworms represent the most "primitive" bilaterians. Some popular theories concerning the origin of the Bilateria will be addressed below incorporating the results of this study on the gastrotrich muscular system.

Several models on the origin of the Bilateria postulate the ancestor to be a vermiform organism (reviewed in Rieger & Ladurner 2001). Acoel flatworms were originally predicted to be the most primitive metazoans according to the Ciliate-Acoel Theory of Hadzi (1963) or, in a different context, as neotenous descendants of the most primitive metazoans according to the Planula-acoel theory of von Graff (1891). Other theories predict the original bilaterian was a macroscopic coelomate organism with a microscopic acoelomate or pseudocoelomate larva (Rieger 1994). While theories postulating Acoela as the most primitive metaozoans have been rejected based on ultrastructural evidence (Smith & Tyler 1985), molecular evidence suggests otherwise (Baguña et al. 2001); consequently, the idea that the original bilaterian was an acoelomate or coelomate organism remains equivocal.

Similarities between gastrotrichs and any coelomate taxa are few; however, similarities become more evident when comparing gastrotrichs to groups that have become secondarily acoelomate. According to Fransen (1980) and Westheide (1985), several groups of microscopic interstitial annelids are derived from macroscopic epibenthic or burrowing forms that experienced evolutionary reductions in body size with subsequent loss of the coelom by expansion of peritoneal cells or the apical ends of muscle cells. In a typical macroscopic coelomate annelid, muscles are generally arranged as outer circular and inner longitudinal sheets surrounding layers of splanchnic musculature. Deriving the gastrotrich muscular system from such a layered organization

is not difficult to postulate using analogies drawn from the evolution of interstitial annelids. In species such as *Apodotrocha* and other dinophilid polychaetes, the musculature underwent reductionary changes in organization from sheet-like monolayers to discontinous band-like elements (Fig. 9.3).

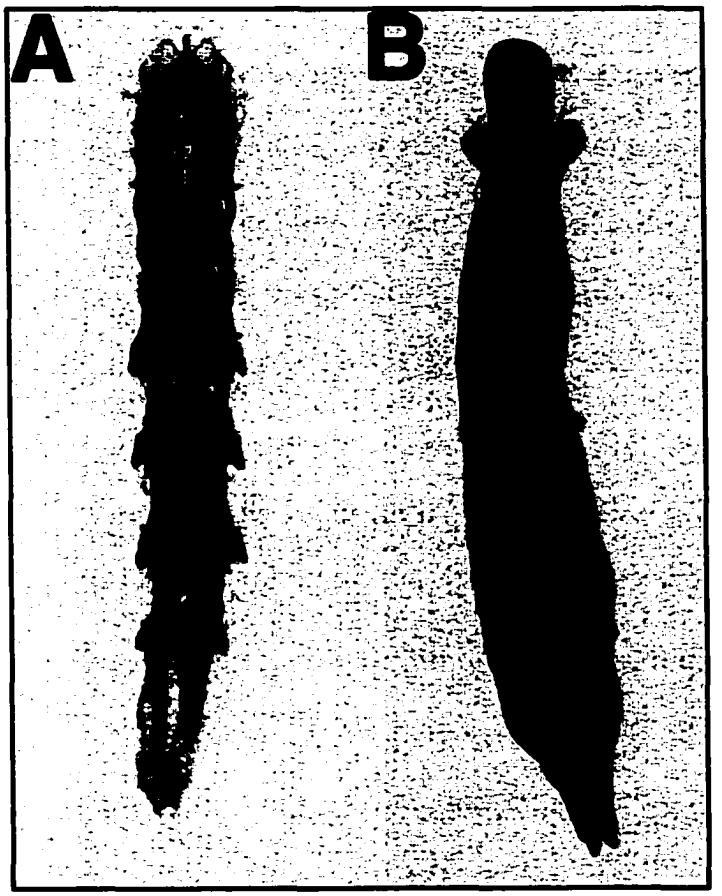



Figure 9.3. Phalloidin-stained interstitial species with band-like muscles. A) *Apodotrocha* sp. (Annelida, Polychaeta) and *Dactylopodola australiense* (Gastrotricha).

Based on the annelid model, a plausible evolutionary origin for the Gastrotricha would involve the secondary invasion of a coelomate ancestor into the interstitial environment leading to evolutionary reductions in organization. Sheet-like muscles once effective for peristaltic locomotion were reduced to discontinuous fiber-type muscles

when the primary means of locomotion changed to ciliary gliding. The ancestral orientation of the muscles was retained (outer circular muscles, inner longitudinal muscles) while new orientations arose (helicoidal muscles, dorsoventral muscles). If this is a plausible hypothesis, primitive gastrotrichs should retain somatic circular fibers surrrounding an inner layer of longitudinal bands arranged radially around the digestive tract. Such a condition is reminiscent of species of *Dactylopodola* (Chapter 3) and *Cephalodasys* (Travis 1983).

A second theory concerns the evolution of acoelomate groups via progeneis of a microscopic larva from a coelomate animal (Rieger 1994). Precocious sexual maturation and evolution of a vermiform body shape may well have led to the origin of the Platyhelminthes (reviewed by Tyler 2001) and other acoelomate groups. Evidence for a progenetic origin of the Gastrotricha may be found in the developmental origin of the musculature. The larval musculature of polychaete annelids and other spiralians is ectodermally derived (as opposed to mesoendodermal derivatives of the $4d_2$ cell) and organized into discrete bands (Andersen 1973; e.g., Fig. 9.4). An analogous situation can be observed in the Gastrotricha where apparently all muscles are ectodermal (meso-ectodermal) in developmental origin according to Teuchert (1968). If the ectodermal origin of muscles is corroborated in more basal species such as those of *Dactylopodola* or *Neodasys*, this could be

Figure 9.4. Phalloidin-stained wholemount of a larva of an echiuran worm showing the band-like muscle arrangement.

further evidence for the origin of gastrotrichs via progenesis. The band-like construction of muscles in gastrotrichs would therefore be plesiomorphic, while the specific muscular topology (circular, longitudinal, etc.) would likely be the result of adaptation to the interstices.

However, the band-like arrangement of muscles in gastrotrichs is also found in several other taxa including Arthropoda (Fig. 9.4A), Gnathostomulida (Tyler & Hooge 2001), and Rotifera (Hochberg & Litvaitis 2000a; Fig. 9.5B), suggesting multiple origins for this type of muscle organization. It is also noteable that band-like muscles do not occur randomly among invertebrates but are most frequently present in microscopic and interstitial forms. Taxa that are proposed to have evolved interstitially like the Gnathostomulida and Gastrotricha may therefore have evolved under similar selective pressures, making it difficult to separate adaptation from phyletic heritage.

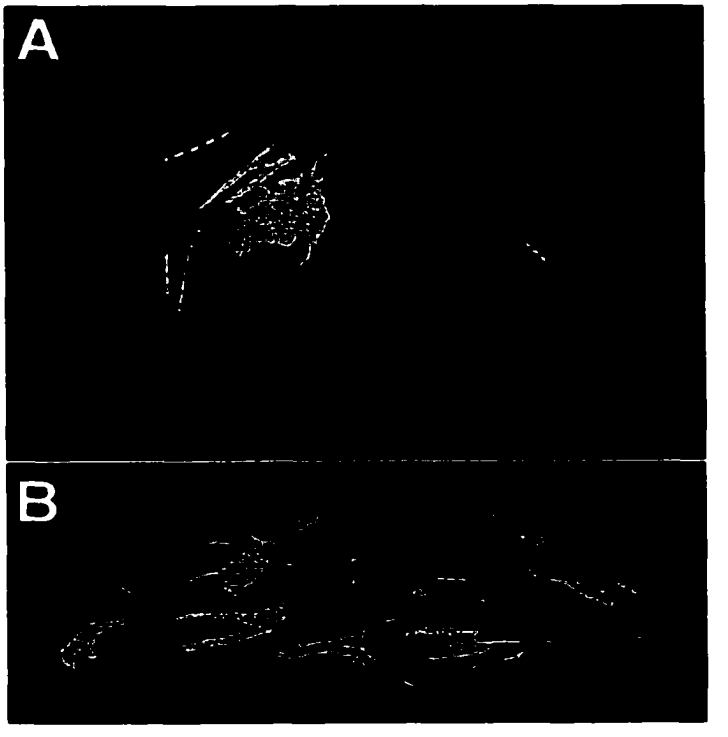



Figure 9.5. Example of interstitial animals with band-like muscles. A) Lateral view of the anterior end of a harpacticoid copepod. B) Lateral view of the rotifer *Philodina* sp.

## Concluding Remarks and Future Directions

Morphological data have established a coherent phylogenetic framework for the Gastrotricha and remain the dominant characters for analysis of evolutionary trends within the phylum. While molecular characterization of gastrotrichs is still in its infancy, preliminary results of Balsamo et al. (2001) agree at least in part with those derived from morphology. The next step toward understanding the origins and relationships of gastrotrichs lies in adding to both data sets.

Additional morphological observations, at the gross anatomical level and the ultrastructural level, are important to increase phylogenetic resolution, especially at lower taxonomic levels. The organization of the muscular system has provided important clues on generic relationships among gastrotrichs, and personal observations suggest that species-level differences may also be abundant. However, as it stands, there are no comparative species-level analyses. This limits confidence in understanding generic and familial relationships which assume *a priori* that all species within a genus are similar (e.g., Hochberg & Litvaitis 2000c, 2001c). Because morphology is often highly plastic at the species level, making phylogenetic work cumbersome, it is recommended that more novel molecular techniques (e.g., mtDNA) be employed to construct species-level phylogenies for comparison.

Future work on gastrotrichs should employ multiple levels of study when addressing the muscular system, including confocal laser scanning microscopy and ultrastructure. It is also suggested that future research on gastrotrichs follow in the footsteps of flatworm biologists who employ a wide variety of techniques (e.g., immunofluorecence, immunogold-gold labelling, cell lineage tracers, molecular

characters, etc.) to understand all levels of organization (e.g., embryology, muscle topology, neurology, spermatology, etc.). Embryological fate map construction should resolve the conflicting origin of mesoderm in both orders of gastrotrichs (reviewed in Hummon 1974). Finally, molecular work involving the search for Hox genes in gastrotrichs should help clarify their phylogenetic position, provided specific Hox gene paralogs such as *Ultrabithorax*/ *Abdominal A* are truly clade- specific synapomorphies as previously suggested (Telford 2001).

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